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13. ABSTRACT *(Maximum 200 Words)*

Male breast cancer is rare, with an incidence rate of 0.5-1/100,000 per year. The objective of this grant is to study male breast cancer cases to estimate the attributable risk of male breast cancer due to *BRCA2* mutations. 170 Caucasian male breast cancer cases are participating, with age at diagnosis ranging from 28-93 years. Of the 166 cases with family history data, 49% have a family history of breast cancer in at least one first- or second-degree relative. Screening for *BRCA2* mutations has been completed for 123 cases. Eleven deleterious mutations were identified in 17 cases, including 6 cases with the common 6174delT mutation. Based on mutations known to be deleterious, the prevalence of *BRCA2* in male breast cancer is 13.8% (17/123) for both population- and clinic-based samples. When only considering population-based samples, the prevalence is 8.5% (8/94). Accounting for the sensitivity of SSCA of approximately 80%, the prevalence of *BRCA2* is 10.6% [(8/94)/0.80] for population-based samples only and 17.2% for all screened samples. Family history is not a good predictor of *BRCA2* mutation status. *BRCA2* mutations appear to be more prevalent in unselected male cases than in unselected female breast cancer cases.

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Susan L. Neuhausen 7-7-00

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Annual Progress Report
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Period: June 16, 1999-June 15, 2000

INTRODUCTION

Breast cancer is a rare disease in men, affecting approximately 0.1% over their lifetime, as compared to 12% in women. However, despite the difference in prevalence, male and female breast cancers are similar in presentation and response to treatment. Risk factors for male breast cancer (MBC) include a positive family history of male or female breast cancer, benign breast disease (primarily gynecomastia), testicular disorders, Klinefelter's syndrome (XXY), and obesity (see reviews in Thomas, 1993; Lynch et al., 1999). The objective of this grant is to characterize the role of *BRCA2* in MBC and to estimate the attributable risk of MBC due to *BRCA2* germline mutations. Currently, 170 Caucasian MBC cases are participating in this research project.

BODY

Our primary goals for the past year included continued ascertainment of additional male breast cancer cases and screening for mutations in the *BRCA1* and *BRCA2* genes.

Technical Objective 1 (tasks 1-4): Ascertainment of male breast cancer cases

Participating individuals. This study was approved by the University of Utah Institutional Review Board. Males with confirmed breast cancer diagnosed between the years 1963 and 2000 have been and are being enrolled in the study (Table 1). The male breast cancer cases from the Utah Cancer Registry, the Wyoming Cancer Registry, the Colorado Cancer Registry, the Idaho Cancer Registry, and the Imperial Cancer Research Fund (ICRF) are population-based. Those from Memorial Sloan Kettering Cancer Center (MSKCC), University of Chicago, and University of Texas Southwestern Medical Center are clinic-based. Those from the internet are self-selected. For the participants from MSKCC and ICRF, DNA samples and minimal questionnaire data were mailed to us. For each participant from the Registries and internet, a 15 ml blood sample was collected. The Registry and internet participants have also been asked to complete a self-administered questionnaire with detailed family history of breast and other cancers, of which 78 are completed and 23 are pending. Of these 105 cases, 11 are now deceased. Diagnoses are verified through medical records when available. The mean age at diagnosis was 61 years with a range from 28-93 years. Family history of breast cancer in first- and second-degree relatives was collected from the majority of participants. DNA was extracted from blood using a Gentra™ kit.

Table 1. Source of male breast cancer cases

Source	# cases	+family history	- family history	Unknown family history
Utah Cancer Registry	65	35	30	0
Idaho Cancer Registry	3	1	2	0
Colorado Cancer Registry	16	5	10	1
Wyoming Cancer Registry	4	1	3	0
ICRF	31	10	21	0
WWW internet site	17	10	6	1
University of Chicago	6	2	2	2
Memorial Sloan Kettering	24	15	9	0
University of Texas	4	3	1	0
Total*	170	82	84	4

* An additional 15 cases, 10 from Colorado, 3 from WWW site, 1 from Wyoming, and 1 from Utah have agreed to participate. We are waiting for consent forms, questionnaires, and blood samples to be returned.

Family history and age at diagnosis. Data for family history of breast cancer in first- or second-degree relatives were available for 161 men. Of the 161 men, 77 had a family history and 84 had no family history. Eight of the men with a family history were diagnosed at less than 41 years of age as compared to no men without a family history. No men with a family history were diagnosed after age 79 years as compared to 6 men without a family history. Mean age at diagnosis was 56.5 years for those with a family history and was 63.9 years for those men without a family history ($p < 0.001$).

Klinefelter syndrome. As part of examining risk factors for MBC, we investigated whether there was an excess of Klinefelter syndrome (an extra X chromosome so that the genotype is XXY). Tissue was unavailable for determining the karyotype of the MBC cases. As a surrogate for karyotyping, we genotyped with two polymorphic markers on chromosome X, DXS102 and the androgen receptor CAG(n). We observed the number of heterozygotes at the markers, which would indicate two X chromosomes. Of the 155 male breast cancer cases for whom genotyping was complete at both chromosome X loci, 2 men (1.3%) appear to have two X chromosomes, suggestive of Klinefelter syndrome. This is a significantly increased rate over the estimated population prevalence of 1-2 per 1,000 men ($p < 0.001$). Our estimate is likely conservative, because we can not distinguish between men homozygous (XXY) and men hemizygous (XY) at these loci on the X chromosome. Other studies have reported that 3-7.5% of male breast cancer cases have Klinefelter syndrome (reviewed in Lynch et al., 1999).

<u>Technical Objective 2 (tasks 5-7): Characterization of loss of heterozygosity (LOH).</u>

The original intent of this objective, designed prior to cloning *BRCA2,* was to classify the MBC cases as likely carrying a *BRCA1* or *BRCA2* mutation based on loss of chromosomal segments in the regions containing *BRCA1* and *BRCA2*. However, *BRCA2* was cloned prior to funding of the

grant, so that we proceeded directly to screening for mutations in the MBC cases. We are still interested in this objective to correlate the LOH results with mutation screening results. However, we are waiting to acquire additional tissue blocks and will perform this objective during this last year of funding. We will only be able to obtain tumor blocks from men participating from the Registries or the internet. Currently, we have tissue blocks from 30 MBC cases, requests to hospitals are pending for 58 cases, blocks are unavailable for 11 cases, and medical release forms still need to be obtained for 10 cases.

Technical Objective 3 : *BRCA1* mutation screening of MBC participants from the Registries and the internet. In a revised Statement of Work, the previous objective 3 to perform fine-structure haplotype construction was replaced with this objective.

Single strand conformational analysis (SSCA) is being used to screen for mutations in *BRCA1*. Primer pairs were designed so that amplicons overlapped and spanned all coding regions and intron/exon boundaries. The amplicon size was less than 250 bp in order to increase the sensitivity to detect mutations. When a variant band was observed, it was sequenced in both directions to identify the actual mutation. There are 45 overlapping amplicons for *BRCA1*. We have completed *BRCA1* mutation screening for 40 male breast cancer cases. No deleterious mutations have yet been detected. We have identified 10 common polymorphisms, which are in linkage disequilibrium, and 4 missense mutations. One variant is currently being sequenced. During the last year of funding, we will complete mutation screening for all DNA samples from MBC cases from the Registries and internet.

Technical Objective 4 (tasks 11-12): Screening for *BRCA2* mutations

SSCA is also being used to screen for mutations in *BRCA2*, using the same procedures as described in Technical Objective 3. There are 73 amplicons for *BRCA2*. When a variant band is observed, it is sequenced in both directions to identify the actual mutation. Mutation screening has been completed for 123 cases. For some of the MBC cases from ICRF and MSKCC, there is insufficient DNA to complete testing for all amplicons. During the last year of funding, we will complete screening for all MBC cases that have enrolled.

Table 2. *BRCA2* mutations identified from 123 MBC cases screened

Mutation	Nucleotide change	Type of mutation	# observations; (%)
IVS2+1G>A	G>A	Splice	1
279delAC	DelAC	Frameshift	1
1002delAA	del AA	Frameshift	1
2158delA	del A	Frameshift	1
4359ins6	ins TGAGGA	Frameshift	1
6174delT	del T	Frameshift	6 –from MSKCC
6175delG	del G	Frameshift	1
8804delA	del A	Frameshift	1
8822insT	ins T	Frameshift	1
9325insA	ins A	Frameshift	2
9481insA	ins A	Frameshift	1
G49L	G>T	Missense	1
S2247G	A>G	Missense	1
T1505A	A>G	Missense	1
T1915M	C>T	Missense	2
A2466V	C>T	Missense - likely polymorphism	1*
D1420Y	G>T	Missense - likely polymorphism	3
N991D	A>G	Missense - likely polymorphism	4
IVS16-14T>C	T>C	Non-coding	1
IVS8+56C>T	C>T	Non-coding	1
IVS2+1G>A	G>A	Non-coding splice	1
203G>A	G>A	Polymorphism	(18%)
3' UTR	A>C	Polymorphism	(22%)
3' UTR	DelT	Polymorphism	(15%)
K1132K	A>G	Polymorphism	(23%)
IVS21-66T>C	C>T	Polymorphism- non-coding	(52%)
K3326X	A>T	Known polymorphism	1
3' UTR	A>G	Likely polymorphism	3
L1522L	G>A	Silent	1*
S646S	C>T	Silent	1
V2171V	C>G	Silent	1*

*Same individual had three variants.

Of the 17 MBC cases with a deleterious frameshift or splice mutation, 6 had no family history, 2 had only a second-degree relative with breast cancer, and 9 had a first-degree relative with breast cancer. Nine of the MBC cases with mutations were from MSKCC and 7 had a family history of breast cancer. Of the 8 population-based samples with a deleterious mutation, 4 had a positive family history.

Technical Objective 5 (task 13): Extending and sampling within families of male breast cancer probands with BRCA2 germline mutations

We are only able to extend families from MBC cases with mutations that were identified in the Registries or on the Internet. The families from MSKCC and ICRF are unavailable. Of the five cases available to extend into their families, two families can be extended and one already has been sampled, two families can not be extended, and one case has two daughters (one of whom is already sampled).

Plans for the final year of funding. Our focus for this last year will be: 1) to complete mutation screening in *BRCA1* and *BRCA2* for the participants from the Registries and the internet; 2) to examine LOH at *BRCA1* and *BRCA2* and correlate the findings with identified *BRCA* mutations, and 3) attempt to extend those families where frameshift mutations are identified.

KEY RESEARCH ACCOMPLISHMENTS

- Collecting the largest single-site set of MBC cases
- Identification of *BRCA2* mutations in MBC cases
- Identifying that Klinefelter syndrome is in excess in MBC
- Determining that a family history of breast cancer is associated with an earlier age at diagnosis

REPORTABLE OUTCOMES:

Neuhausen S, Godwin A, Gershoni-Baruch R, Schubert E, Garber J, Stoppa-Lyonnet D, Olah E, Csokay B, Serova O, Lallo F, Osorio A, Stratton M, Offit, K, Boyd J, Caligo A, Scott R, Schoefield A, Teugels E, Cannon-Albright L, Bishop T, Benitez J, King MC, Ponder B, Weber B, Devilee P, Borg A, Narod S, Goldgar D: (1998) Haplotype and phenotype analysis of nine recurrent *BRCA2* mutations in 111 families: results of an international study. Am J Hum Gen., 62:1381-1388.

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Neuhausen S: (1999) Genetic epidemiology of breast, ovarian and endometrial cancers. In: Familial and Hereditary Cancer in Women. (S. Mancuso, S. Pecorelli, eds.) Poletto Editore srl,. pp 47-63.

Neuhausen SL: (2000) Prevalence of BRCA2 mutations in male breast cancer cases. Second ERA of Hope, DAMD Meeting.

CONCLUSIONS

Male breast cancer is a relatively rare disease, as shown by our difficulty in collecting a large number of living cases for this study in a short period of time. Thus, the cases are prevalent cases rather than incident cases. Time from diagnosis to enrollment varied from less than 12 months to 30 years. The MBC cases with a family history of breast cancer were diagnosed, on average, 13 years earlier than those without a family history.

BRCA2 mutation screening has been completed on 123 MBC cases. Seventeen known deleterious mutations were identified (frameshift mutations that caused premature protein termination and a splice mutation) for a prevalence of 13.8%. Nine of the mutation carriers were from clinics, including 6 MBC cases with the 6174delT mutation, a common mutation in individuals of Ashkenazi Jewish descent. When excluding the cases with the founder 6174delT mutation, the prevalence of *BRCA2* in MBC is 9.4%. When including only the population-based samples, the prevalence of *BRCA2* is 8.5%. Accounting for the sensitivity of SSCA of approximately 80%, the prevalence is 10.6% [(8/94)/0.80] for population-based samples only and 17.2% for all screened samples. Family history is not a good predictor of *BRCA2* mutation status. *BRCA2* mutations appear to be more prevalent in unselected MBC cases than in unselected female breast cancer cases.

This research is ongoing. We will continue to screen for mutations in *BRCA1* and *BRCA2* for male breast cancer cases who have not yet been tested. We will investigate whether lifestyle and medical factors, e.g., weight, smoking, gynecomastia, appear to be associated with male breast cancer.

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Thomas DB: Breast cancer in men. Epidemiologic Reviews 15:220-231, 1993.

APPENDIX

Manuscripts (reportable outcomes)

Prevalence and Characterization of *BRCA2* in Male Breast Cancer Cases
DAMD17-96-6266

Principal Investigator: Susan L. Neuhausen

Annual Report – July 2000

Appendix - Cited manuscripts

Am. J. Hum. Genet. 62:1381–1388, 1998

Haplotype and Phenotype Analysis of Nine Recurrent *BRCA2* Mutations in 111 Families: Results of an International Study

Susan L. Neuhausen,[1] Andrew K. Godwin,[2] Ruth Gershoni-Baruch,[4] Elizabeth Schubert,[5] Judy Garber,[6] Dominique Stoppa-Lyonnet,[7] Edith Olah,[8] Bela Csokay,[8] Olga Serova,[9] Fiona Lalloo,[10] Ana Osorio,[11] Michael Stratton,[12] Kenneth Offit,[13] Jeff Boyd,[14] M. Adelaide Caligo,[15] Rodney J. Scott,[16,*] Andy Schofield,[17] Eric Teugels,[18] Manfred Schwab,[19] Lisa Cannon-Albright,[1] Timothy Bishop,[20] Douglas Easton,[21] Javier Benitez,[11] Mary-Claire King,[5] Bruce A. J. Ponder,[22] Barbara Weber,[3] Peter Devilee,[23] Åke Borg,[24] Steven A. Narod,[25] and David Goldgar[10]

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Summary

Several *BRCA2* mutations are found to occur in geographically diverse breast and ovarian cancer families. To investigate both mutation origin and mutation-specific phenotypes due to *BRCA2*, we constructed a haplotype of 10 polymorphic short tandem-repeat (STR) markers flanking the *BRCA2* locus, in a set of 111 breast or breast/ovarian cancer families selected for having one of nine recurrent *BRCA2* mutations. Six of the individual mutations are estimated to have arisen 400–2,000 years ago. In particular, the 6174delT mutation, found in ~1% of individuals of Ashkenazi Jewish ancestry, was estimated to have arisen 29 generations ago (1-LOD support interval 22–38). This is substantially more recent than the estimated age of the *BRCA1* 185delAG mutation (46 generations), derived from our analogous study of *BRCA1* mutations. In general, there was no evidence of multiple origins of identical *BRCA2* mutations. Our study data were consistent with the previous report of a higher incidence of ovarian cancer in families with mutations in a 3.3-kb region of exon 11 (the ovarian cancer cluster region [OCCR]) ($P = .10$); but that higher incidence was not statistically significant. There was significant evidence that age at diagnosis of breast cancer varied by mutation ($P < .001$), although only 8% of the variance in age at diagnosis could be explained by the specific mutation, and there was no evidence of family-specific effects. When the age at diagnosis of the breast cancer cases was examined by OCCR, cases associated with mutations in the OCCR had a significantly older mean age at diagnosis than was seen in those outside this region (48 years vs. 42 years; $P = .0005$).

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Introduction

The isolation of *BRCA1* (Miki et al. 1994) and *BRCA2* (Wooster et al. 1995; Tavtigian et al. 1996), two genes predisposing to early-onset breast cancer and ovarian cancer, has resulted in rapid identification of a large number of families with mutations in these genes (Breast Cancer Information Core) (Couch et al. 1996*b*; Szabo and King 1997). Although both genes exhibit a large number of distinct mutations, several mutations have been found to recur in a number of independently as-

certained families of apparently diverse geographical origin, as well as in families largely confined to a single population.

Genes responsible for inherited cancer, like many other disease genes, have been associated with a wide diversity of expression. This is seen not only in variability in the age at diagnosis of cancer but also in the anatomical site at which the tumor originates. More important, at least from the clinical perspective, is the degree to which specific mutations and accompanying genetic backgrounds influence the expression of *BRCA2* in terms of site and age at diagnosis. For *BRCA2*, Gayther et al. (1997) have provided evidence that mutations in an ~3.3-kb nucleotide region of exon 11 (denoted the "ovarian cancer-cluster region" [OCCR]) are associated with a higher incidence of ovarian cancer relative to breast cancer. In that study, this was highly significant, with an ovarian: breast cancer ratio of 11:45 inside, and 22:282 outside, the OCCR. In the present studies, four of the mutations examined were within the OCCR, whereas the other five were outside this region. This allowed us to examine, with the present data set, the OCCR hypothesis.

In a previous paper (Neuhausen et al. 1996*b*), we analyzed six recurrent *BRCA1* mutations for haplotype conservation, over a 3-Mb segment containing the *BRCA1* gene, using nine STR markers. We also investigated the relationship between the position of the mutation and the phenotype (in terms of both age at diagnosis of breast cancer and proportion of ovarian cancer) of the families carrying each mutation. In the present article, we have undertaken a similar study of recurrent *BRCA2* mutations, addressing both mutation origin and the relationship between mutation and phenotype. To do this, we constructed a haplotype of 10 polymorphic STR markers flanking the *BRCA2* locus in a set of 111 families (selected to contain one of nine *BRCA2* mutations that had been identified a minimum of three times) and analyzed the phenotype associated with each mutation. For five mutations for which sufficient haplotype data existed, we estimated the age of the mutation, using a modified version of our mathematical model developed for our *BRCA1* analysis.

Subjects and Methods

Family Ascertainment

Families with one of the nine mutations were from 24 centers located in 13 countries in Europe and North America. The families had been previously ascertained for a variety of reasons, including research studies, directed screening of case series of ovarian or male breast cancer, or attendence at a cancer genetics clinic. Appropriate informed consent was obtained from all participants. When possible, pedigree information was obtained, although for several centers, no such history was available and, for other centers, only a limited family history could be obtained. All cases of breast and ovarian cancer reported in the pedigree were included in the study, with the exception of cases who were known to not carry the *BRCA2* mutation segregating in the family. No independent verification of diagnosis was obtained, and, for a small proportion of cases, age at diagnosis was not available.

Samples for the 982del4 mutation were from the United States and France; those for 2041insA, from Germany, Canada, and the United States; those for 3034del4, from Belgium, Canada, Spain, France, Switzerland, Italy, and the United States; those for 4486delG, from Sweden; those for 5573insA, from the Netherlands; those for 6174delT, from Canada, France, Israel, Hungary, Sweden, the United Kingdom, and the United States; those for 6503delTT, from Belgium, the Netherlands, Sweden, and the United Kingdom; those for 9254del5, from France and Spain; and, those for 9326insA, from Hungary, Sweden, and the United Kingdom.

Genotyping of 13q Markers

Genotyping was performed at four centers. The families collected by the University of Washington in Seattle, the National Institute of Oncology in Budapest, and the Fundacion Jimenez Diaz in Madrid were genotyped in their respective laboratories; all other families were genotyped in the Genetic Epidemiology Laboratory at the University of Utah. At all centers, the same five DNA samples were used as controls, and a similar protocol was followed. All 10 markers genotyped were STR loci assayed by PCR, with standard procedures. All the results in the tables are from analyses of all 10 markers. For all mutations except 6174delT, allele frequencies used in the likelihood calculations were as reported in Genome Database, from typings of ~80 independent CEPH chromosomes. For analysis of family samples of Ashkenazi Jewish ancestry carrying the 6174delT mutation, we estimated marker-allele frequencies from the haplotype data of the non–mutation-bearing chromosomes. In all cases, allele sizes were matched according to the genotype of CEPH reference individual 1347-02, who was used as a control on each gel. The genetic map assumed for the haplotype analyses was derived from physical-mapping data (Couch et al. 1996*a;* S. L. Neuhausen, unpublished data), under the assumption that 1.5 cM = 1 Mb. Note that this rate is higher than the usual 1:1 ratio assumed as a genomewide average; this was done to ensure that the total distance of the map was in agreement with that of the published genetic map (Dib et al. 1996). None of the markers were located intragenic to *BRCA2*. The assumed map order and dis-

Table 1

Summary of STRs Used in Haplotype Analysis

Marker	Position (cM)	No. of Alleles	Heterozygosity[a] (%)	Size (Frequency) of Common Allele (bp)	Genotype of 1347-02 (bp)
D13S290	2.70	6	46	176 (.71), 190 (.11), 188 (.11)	190/176
D13S1444	1.35	9	80	167 (.41), 169 (.24), 177 (.11)	177/167
D13S1700	1.20	18	89	308 (.12), 312 (.09), 258 (.09)	320/254
D13S260	1.00	9	78	163 (.41), 161 (.13), 171 (.09)	163/161
D13S1699	.72	6	67	150 (.54), 146 (.37)	156/146
D13S1698	.63	10	63	152 (.35), 154 (.30)	168/160
BRCA2	.0				
D13S171	−.60	6	72	241 (.32), 231 (.32), 227 (.25)	231/231
D13S1695	−.96	11	79	245 (.37), 247 (.23)	249/235
D13S310	−2.10	5	70	146 (.40), 144 (.24), 140 (.24)	146/146
D13S267	−3.12	6	69	148 (.44), 160 (.29), 154 (.17)	160/148

[a] Determined from genotyping of 80–100 chromosomes.

tances and the descriptions of the markers used are given in table 1.

When possible, haplotypes associated with each mutation were inferred from multiple samples of related individuals within each kindred known to have the same mutation; otherwise, multilocus genotypes were compared. When haplotypes could not be determined with certainty, all possible haplotypes (to a maximum of 64) consistent with the observed multilocus genotypes were considered in the likelihood analysis, in a manner analogous to the phase calculations in multipoint linkage analysis.

Analysis of Haplotype Data

The estimation of the age of the mutations was performed by use of the same statistical model that had been used in our previous analysis of *BRCA1* (Neuhausen et al. 1996*b*), with several minor modifications. In brief, the joint likelihood of the *BRCA2* haplotypes (or all possible haplotypes from families with a given mutation, relative to a presumed ancestral haplotype) is written as a function of the recombination fraction between the disease and each marker; the number of generations, *G*, since the mutation arose; and the mutation rate and allele frequencies at each marker locus. The marker D13S1700 was assumed to have a higher mutation rate (.01) than the other markers (.002 for a tetranucleotide repeat and .0006 for a dinucleotide repeat), on the basis of both the large number of alleles and the observation of mutations within families. We also included another parameter, μ_D, the proportion of families with an independent mutation identical to that of the presumed ancestral haplotype. This parameter is analogous to genetic heterogeneity in standard linkage analysis and can be estimated from the data.

The method of maximum likelihood was used to find the value of *G* that, among families with identical mutations, best fitted the pattern of haplotype sharing at the 10 marker loci. Approximate support intervals for the age of each mutation were calculated by finding the value of *G* on either side of the most likely value that had a ⩾10-fold decrease in likelihood. A test for heterogeneity of mutation origin was performed by comparing the likelihood at the maximum-likelihood estimates of *G* and μ_D with the analogous likelihood, assuming $\mu_D = 0$. Each generation is estimated to be 20 years.

Analysis of Phenotype Data

For each mutation, the number of families with that mutation, the number of female and male breast cancer cases, and the number of ovarian cancer cases were tabulated. To partially counter any effects of ascertainment of those directed-screening cases of breast and ovarian cancers, we also examined the data only in those families in which there were at least three cases of cancer, where a case is defined as a female breast cancer at age <60 years, an ovarian cancer, or a male breast cancer. In this second tabulation, only cases of female breast cancer at age <60 years were counted in the breast cancer results; this was done in order to increase the probability that they were associated with the *BRCA2* mutation segregating in the family.

To test for heterogeneity in the proportion of affected individuals who had ovarian cancer, as a function of whether the mutation associated with a given family was inside or outside the hypothesized OCCR, a randomization test was performed. Specifically, random permutations of families with the nine mutations were performed, in which the number of families with each mutation was kept equal to that present in the actual data set. After this permutation step, the mutations were grouped according to their location respective to the OCCR. Each such permutation resulted in a different

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Table 2

Results of Haplotype Analysis of Nine Mutations

Mutation	No. of Families[a]	No. of Countries[b]	Core Haplotype at					Consistency Index[c]	G	1–LOD Interval
			D13S260	D13S1699	D13S1698	D13S171	D13S1695			
982del4	5 (3/2)	2	161	146	154	231	253	5/5	18	(4–43)
2034insA	5 (3/2)	3	163	150	166	241	247	3/5	36	(13–64)
3034del4	11 (4/7)	7	163	146	154	227	245	2/11	80	(46–134)
4484delG	4 (0/4)	1	169	156	166	241	247	4/4	Not calculated	
5573insA	3 (3/0)	1	165	146	154	227	245	2/3	Not calculated	
6174delT	69 (22/47)	7	161	146	152	239	251	45/69	29	(22–38)
6503delTT	7 (5/2)	4	163	150	158	227	245	3/5	52	(24–98)
9254del5	3 (2/1)	2	163	150	154	231	X	2/3	Not calculated	
9326insA	4 (1/3)	3	171	152	152	231	245	2/4	Not calculated	

[a] Data in parentheses are number of families in which haplotypes could be determined/number of families for which only multilocus genotype data were available.

[b] For names of countries, see the Subjects and Methods section.

[c] Number of samples/families consistent with core haplotype for all five markers listed.

2×2 table with an associated χ^2 statistic calculated in the standard fashion. The χ^2 statistic associated with the observed aggregation of cases and mutations was compared with those calculated from 2,000 random permutations of families and mutations. The rank of the observed χ^2 statistic among those from 2,000 replicates is the nominal *P* for testing the association between the prevalence of ovarian cancer and a specific mutation. The S-Plus package (StatSci) was used to perform the randomization test. Phenotypic analysis of age at diagnosis, among mutations, was performed by the T-TEST, GLM, and VARCOMP procedures of the SAS statistical analysis package.

Results

Haplotype Analysis and Age of Mutations

The mutations described in this report span the *BRCA2* gene and are small insertions or deletions that cause protein truncation. In table 2, the mutations are characterized as to the number of families studied, the numbers of genotypes and haplotypes obtained, and the geographic diversity (as based on the number of countries from which samples were contributed). The most common haplotype associated with each of the nine mutations studied, as well as the estimated *G*, support interval, and estimated heterogeneity for those mutations with at least five haplotypes to analyze are also shown in table 2. Although the estimation of the ages of the mutations incorporated data from all 10 markers, we report the consensus haplotype at the six markers closest to *BRCA2*, since, in many cases, the haplotype beyond these markers was difficult to determine. For four of the five mutations examined, the estimated fraction of families in which cancer was due to an independent mutational event was 0; for 6503delTT, the estimated proportion was .11, which is not significantly different from 0. For 6174delT, the 1-LOD upper bound for the proportion attributable to one (or more) independent identical mutations was .06. In all cases, there was no significant evidence of mutational heterogeneity, indicating that, for each mutation studied, all families with the mutation represent derivations from a single ancestral haplotype on which the mutation arose. The estimates of *G* are based on assumptions about mutation rates and recombination rates and therefore may be more appropriately considered as relative indications of time since the mutation originated, rather than as absolute values. We estimate the 982del4, as an example, to have occurred relatively recently—that is, 18 generations ago (1-LOD support interval 4–43), or ~360 years ago (1-LOD support interval 80-860 years).

Association between Phenotypic Variation and Mutations

A summary of the number of cases of breast and ovarian cancers and the ages at diagnosis of the breast cancer cases, stratified by *BRCA2* mutation type, is shown in table 3, for all families with all breast cancer cases and for those "high-risk" families (as described in the Subjects and Methods section) that have breast cancer cases diagnosed at age <60 years. There was significant variation in age at diagnosis among the nine mutations tested when all cases in all families were considered ($P = .0007$, by nested ANOVA), as well as when the analysis was restricted to high-risk families and cases diagnosed at age <60 years ($P = .015$), although only ~8% and ~6%, respectively, of the variance was explained by individual mutation. In both analyses, there was no evidence of significant variation between families, for any mutation group, and the variance due to this effect was estimated to be zero in both cases.

Table 3

Summary of Phenotypic Data Associated with Mutation

	All Families[a]				Families with ≥3 Cases[b]			
		No. of Cancer Cases				No. of Cancer Cases		
Mutation	No.	Female Breast (Age [years])	Ovarian	Male Breast	No.	Female Breast at Age <60 Years (Age [years])	Ovarian	Male Breast
982del4	5	25 (41)	1	4	4	20 (38)	1	4
2041insA	5	16 (41)	4	5	4	11 (39)	3	5
3034del4	11	37 (42)	6	2	9	33 (42)	5	2
4486delG	5	16 (48)	0	3	1	6 (44)	0	0
5573insA	3	5 (47)	7	0	2	2 (40)	7	0
6174delT[c]	67	119 (49)	29	12	22	60 (46)	12	8
6503delTT	7	20 (44)	12	1	6	18 (44)	12	1
9254del5	3	16 (48)	3	3	3	11 (43)	3	3
9326insA	4	9 (34)	0	2	1	3 (35)	0	1
Total	110	263 (45.6)	62	32	52	164 (42.7)	43	24

[a] Includes all families on which at least some phenotypic information was available. Breast cancer tabulation contains all cases of breast cancer, regardless of age, as well as those cases for which age at diagnosis is unknown.

[b] Families with at least three cases of cancer, where a case is defined as a female breast cancer at age <60 years, an ovarian cancer, or a male breast cancer. Only the cases of female breast cancer at age <60 years are included in the results.

[c] In 13 families obtained from a consecutive series of Ashkenazi Jewish ovarian cancer patients tested only for the 6174delT mutation, the ovarian cancer proband was omitted from this table and subsequent analyses; however, the proband was used in determining whether the family had three or more cases.

Examination of the OCCR

The randomization test described in the Subjects and Methods section was used to examine possible differences in the relative proportions of cases of breast and ovarian cancers, for mutations inside and outside the OCCR. These results are shown in table 4. It is clear that there is a higher proportion of ovarian cancer cases associated with families with mutations in the OCCR region, although this difference is not significant for either the complete data set ($P = .12$) or the high-risk subset ($P = .11$). The odds ratio for the entire set of families is 2.1. Interestingly, when we examined the age at diagnosis of the breast cancer cases in terms of OCCR status, we found that most of the age-at-onset variation between mutations could be ascribed to the location relative to the OCCR. This difference, of older age at onset for the OCCR region, was highly significant, both for the nested analysis of variance with between-family variation used as the error term and by ordinary t-test. Because the 6174delT mutation group was the largest and had the oldest age at onset, we also performed the analysis of age at onset and OCCR again, without this group. When we removed the cases with a 6174delT mutation, the effect of the mutation location in the OCCR is still present but is not significant ($P = .09$).

Discussion

In this paper, we have analyzed genotypic and phenotypic data from a series of breast cancer families and from isolated cases with one of nine recurrent mutations in the *BRCA2* gene. These data appear to include both population-specific sequence variants, as well as those found in more geographically diverse populations of northern European Caucasian ancestry. The mutation with the oldest estimated age, 3034del4, was found in the most diverse set of samples (except for the 6174delT mutation in the Ashkenazi population), both in multiple centers in the same country and in seven different countries. For the mutations studied, the multiple instances of specific mutations generally appear to represent founder effects many generations in the past, rather than independent mutational events. This is in contrast to the *BRCA1* mutations—4184del4, Arg1443ter, and 185delAG—which, on the basis of the multiple origins of these mutations, may represent hot spots (Neuhausen et al. 1996*b*).

The 4486delG mutation has been reported only in Scandanavia (Håkansson et al. 1997). For this study, there were too few haplotypes to determine the age of the mutation. However, all four samples (three from sporadic male breast cancer cases and one large breast cancer family) genotyped with this mutation appeared to share a conserved haplotype over an ~3-cM interval containing the *BRCA2* locus. A similar pattern was observed in the three Dutch families carrying the 5573insA mutation. The 9254del5 mutation has been identified only in two French families of Catalan origin and in a single Spanish family also from this region. The three families share a conserved haplotype over an ~2-cM region spanning the *BRCA2* locus. These three families have different phenotypes, with one family having three cases

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Table 4

Examination of OCCR

Mutation Location	All Families[a]				Families with ≥3 Cancer Cases[b]			
		No. of Cancer Cases				No. of Cancer Cases		
	No.	Female Breast (Age [years])	Ovarian	Male Breast	No.	Female Breast at Age <60 Years (Age [years])	Ovarian	Male Breast
OCCR$^-$	82	160 (48.0)	48	17	31	88 (44.9)	31	9
OCCR$^-$	28	103 (41.9)	14	16	21	76 (40.3)	12	15

[a] As defined in table 3. For age at diagnosis, $P < .0001$; for breast cancer versus ovarian cancer, $P = .12$.

[b] As defined in table 3. For age at diagnosis, $P < .0005$; for breast cancer versus ovarian cancer, $P = .11$.

of male breast cancer and four cases of female breast cancer, a second family having three cases of ovarian cancer, and a third family having eight site-specific cases of female breast cancer.

By contrast, the 3034del4 mutation has been found in families in seven different western European and North American countries (Belgium, Canada, France, Italy, Spain, Switzerland, and the United States). There was a considerable amount of haplotype diversity among the 11 families examined, accounting for the large value of the estimated age. Although our analysis failed to find significant statistical evidence of multiple independent origins for this mutation (the maximum-likelihood estimate for the proportion due to independent mutation is 0), given the limited number of families available for analysis, statistically we could not rule out the possibility that there were independent mutations for as many as half the families. This mutation is in a region that may be a hot spot for such deletions. Another 4-bp deletion, located only 2 bp downstream, has been reported in five families thus far, and a 2-bp deletion located 4 bp downstream has been reported once (Breast Cancer Information Core).

Of particular interest is the 6174delT mutation found in high frequency in the Ashkenazi Jewish population. Along with the two *BRCA1* mutations (185delAG and 5382insC), it has been estimated that 1 in 50 Ashkenazi Jewish individuals carry one of these three mutations (Struewing et al. 1995, 1997; Oddoux et al. 1996; Roa et al. 1996). These mutations account for ~30% of early-onset breast cancer (Neuhausen et al. 1996*a;* Offit et al. 1996; Tonin et al. 1996) and for as much as 60% of all ovarian cancer in this population (Abeliovich et al. 1997). On the basis of our analysis of haplotypes and genotypes of 69 families with the 6174delT mutation, we estimate that the mutation originated ~29 generations ago (1-LOD support interval 22–38). The corresponding analysis for the age of the *BRCA1* 185delAG, on the basis of our original set of 18 families with this mutation, resulted in an estimate of 46 generations (1-LOD support interval 22–82) and suggested that the cases in ~90% of the families are due to the presumed ancestral Jewish mutation (an estimate reflecting the fact that two families of non-Jewish ancestry were part of the sample). Thus, the 6174delT mutation appears to have originated more recently. Support for the more recent origin of the 6174delT mutation comes from examination of these mutations in 44 non-Ashkenazi Jewish patients. One Iraqi patient had a 185delAG mutation, and none had a 6174delT mutation (Abeliovich et al. 1997). Sher et al. (1996) also reported a 185delAG mutation in an Iraqi Jew, suggesting that this mutation has an origin earlier than that of the 6174delT mutation. More recently, an additional three *BRCA1* 185delAG mutations have been identified, in a sample of 639 Iraqi Jews (Bar-Sade et al. 1997), but, to our knowledge, the 6174delT mutation has never been found outside the Ashkenazi Jewish population.

Our analysis was consistent with the finding by Gayther et al. (1997)—that is, that there is a higher incidence of ovarian cancer relative to breast cancer associated with the OCCR; however, this higher incidence was not statistically significant. One possible reason for the difference between the significance presented here and that reported by Gayther et al. (1997) could be the ill-defined 5′ end of the OCCR. The 3034del4 mutation is on the 5′ border of the OCCR, as defined by Gayther et al. (1997), and its exclusion, rather than inclusion in the OCCR, could have an affect on the analysis.

Among the mutations, there were significant differences associated with age at diagnosis of breast cancer. Much of the variation was associated with mutation location relative to the OCCR. However, when we removed the cases with a 6174delT mutation, the effect of the mutation location in the OCCR, although still present, was not significant. The later age at onset of breast cancer in the cases with the 6174delT mutation could be due to ease of screening families for this common mutation. However, the age effect is still present in those families with three or more cancer cases who would likely be screened in any testing program, suggesting that mutations within the OCCR and/or, more specifically, the 6174delT mutation do confer a later age at onset of breast cancer. On the basis of previous studies

of two common mutations, there is a suggestion that mutations in the OCCR are less penetrant for breast cancer at a younger age. In the Icelandic studies of the 999del5 mutation, which is outside the OCCR, 28% of Icelandic breast cancer cases of age <40 years carry this mutation, which has a population prevalence of 0.50%. In contrast, for the 6174delT mutation, which is within the OCCR, 8% of Ashkenazi Jewish breast cancer cases of age <40 years carry this mutation, which has a population prevalence of 1.2%. Therefore, with a prevalence twofold higher for the 6174delT mutation, there is a large difference, in comparison with the Icelandic mutation, for age at onset of breast cancer, suggesting lower penetrance at age <40 years.

As a first step in mutation detection, comparison of an observed haplotype in a family examination of haplotypes can be useful to identify common mutations. In addition to this set of haplotypes for recurrent mutations, we are also constructing a haplotype database of any mutations, so that others can compare their haplotypes (for further information, please contact S.L.N.). A haplotype database of Dutch mutations is available from a Leiden University Medical Center Department of Human Genetics Website. Since multiple families with identical mutations on identical genetic backgrounds can be ascertained, this will allow us to better elucidate additional genetic and environmental factors that contribute to the observed variation in phenotype. Similarly, studies of families with identical mutations but with different origins will allow us to examine better the possible effect of genetic modifier loci. A copy of the revised version of the haplotype-analysis program is available, on request, from D.E.G.

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Electronic-Database Information

URLs for data in this article are as follows:

Breast Cancer Information Core, http://www.nchgr.hih.gov/intramuraL_research/Lab_transfer/Bic

Leiden University Medical Center Department of Human Genetics ("Haplotypes carrying *BRCA1* mutations found repeatedly in the Dutch population"), http://ruly70.medfac.leidenuniv.nl/~devilee/hapover.htm

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Pathobiologic Characteristics of Hereditary Breast Cancer

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Patients with hereditary breast cancer (HBC) present at a young age with breast cancers that show adverse pathological characteristics such as high nuclear grade, negative hormone receptor status, and high proliferation indices. Surprisingly, the clinical course has been reported to be comparable or improved compared with patients with nonhereditary breast cancer (non-HBC). To determine whether there are any molecular markers that might help explain this paradox between pathologically aggressive neoplasms in patients with HBC and the lack of extreme clinically aggressive disease, we studied several molecular parameters in a group of 34 breast cancer patients with mutations in either the BRCA1 or BRCA2 tumor suppressor genes and compared them with a group of 20 breast cancer patients with non-HBC. In general, patients with HBC had tumors that were of higher nuclear grade, contained a higher population of proliferating cells, showed increased expression of DNA topoisomerase II-alpha (topo II-alpha), lacked hormone receptors, and were more likely to show immunopositivity for the *p53* tumor suppressor gene. Additionally, tumors from patients with HBC showed a decreased angiogenesis compared with controls. The decreased angiogenesis and the elevated expression of topo II-alpha (an anticancer drug target) may, in part, explain the lack of correlation between clinical course and histological characteristics in patients with HBC. HUM PATHOL 29:1140-1144.

Key words: hereditary breast cancer, BRCA1, BRCA2, immunohistochemical staining, DNA topo II-alpha.

Abbreviation: HBC, hereditary breast cancer.

Breast cancer is the leading malignancy in women and the second most common cause of cancer-related deaths in the United States.[1] Observations of a family history of breast cancer with an early age of onset spurred research into the investigation of specific genes that may be responsible for the development of this disease. As a result, BRCA1 and BRCA2, the two genes that appear to confer susceptibility to the development of breast carcinoma, have been isolated and characterized. The BRCA1 gene has been mapped to chromosome 17q12-21, and the BRCA2 gene has been mapped to chromosome 13q12-13.[2,3] Together, these two genes probably account for the majority of hereditary breast cancer (HBC), or 5% to 10% of all breast cancers.[4,5]

The clinical aspects and the pathological characteristics of the neoplasms in patients with HBC have not been widely studied. From the limited data available, it appears that patients with HBC may have a better than or similar prognosis to patients with sporadic tumors.[6-8] This result is surprising because several studies have indicated that breast cancers arising in patients with HBC have pathological characteristics such as high nuclear grade, high proliferation indices, absent hormone receptor status, and increased p53 immunopositivity; features that are usually associated with more aggressive disease.[7,9-13]

In an effort to understand more fully this apparent paradox between relatively favorable clinical course and poor pathological indicators, we evaluated the pathological characteristics of breast carcinoma in 21 patients with known BRCA1 mutations and in 13 patients with known BRCA2 mutations and compared them with the pathological characteristics observed in breast carcinoma from 20 patients with non-HBC. The patients selected for comparison were consecutive cases obtained from a single institution and were not matched to the case groups. Several new histological parameters that may have important prognostic implications in breast cancer, and have not been previously studied in this group of tumors, such as DNA topoisomerase II-alpha and tumor microvessel density, have been evaluated.[14,15]

MATERIALS AND METHODS

Patient Characteristics

Breast cancer tissue was available from 21 patients with BRCA1 mutations (one patient had metachronous, bilateral breast cancers, and consequently, there were a total of 22 cases), and from 13 patients with BRCA2 mutations, representing nine BRCA1 families and six BRCA2 families (Table 1). Each identified mutation was unique, with the exception of kindreds 1001 and 2301.[16,17] Germline mutations were identified by full genomic sequencing for 20 of 21 (95%) of BRCA1 patients and 10 of 13 (77%) of BRCA2 patients. The other cases were included based on a high lod score and shared haplotype among breast cancer cases (Table 1). A group of 20 sporadic cases of breast cancer were retrieved from the surgical pathology files at the University of Utah and were not matched to the BRCA1 or BRCA2 cases. The genotype status of the patients was blinded to the reviewing pathologist. All

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TABLE 1. Mutations

Kindred	No. of Patients	Mutation
BRCA1		
1001	1	SP-FS, IVS 5 (−11, T > G, 59 bp ins)
1901	1	FS, 188 del 11
2035	4	Del, 14 Kb
2082	5	NS, Gln 1313 ter
2099	2	MS, Met 1775 Arg
2301	3	SP-FS, IVS 5 (−11, T > G, 59 bp ins)
2305	3	FS, 2982 del 5
2331	1	Linked
2373	1	FS, 3875 del 4
BRCA2		
107	3	FS, 277 del AC
1018	2	FS, 982 del 4
2044	3	FS, 4766 del 4
2327	2	lod score 1.92
2367	2	SP, IVS 2 (+1, G > A)
2388	1	lod score 0.92

Abbreviations: SP, splice site; FS, frame shift; NS, nonsense; MS, missense; IVS, intervening sequence; del, deletion; ins, insertion.

slides were reviewed to confirm the diagnosis and given a modified Bloom-Richardson score.[18] The use of human tissue for this work was approved by the Institutional Review Board at the University of Utah.

Chemicals and Antibodies

The source of the chemicals and antibodies used were as described.[14] In addition, antibodies against the von Willebrand factor and p53 (clone DO-7) were from DAKO (Carpinteria, CA).

Immunohistochemical Staining and Interpretation

Immunohistochemical staining of histological sections prepared from human breast cancers was performed as described in detail elsewhere.[14] Briefly, slides were deparaffinized and heated (except for HER2/neu, which does not require the heating step) in 10 mmol/L sodium citrate (pH 6.0) for 30 minutes in a microwave oven. After cooling, immunohistochemical staining was performed with the use of a Ventana 320 automated immunohistochemical stainer in accord with the manufacturer's instructions. Detection was with a secondary mouse anti-immunoglobulin linked to biotin followed by incubation with streptavidin linked to horseradish peroxidase. Color development was accomplished with diaminobenzidine as the chromogen.

The dilutions of the antibodies used in immunohistochemical staining were as follows: topo II-alpha, 1:500; MIB1, 1:40; estrogen and progesterone receptors, 1:60; Her2/neu (c-erb-2): 1:800; Factor VIII: 1:1600; p53, 1:80.

Topo II-alpha and MIB1 were expressed as the topo II-alpha or MIB1 index, respectively. This was performed as described and represents the percent of positive staining cells.[14] Evaluation of p53 expression was performed in a similar fashion. At least 500 tumor cells were counted, and the number of positive p53 staining cells was determined. Evaluation of p53 immunostaining has not yet been standardized. Authors have used as little as 1% to greater than 20% cell positivity as a positive interpretation, suggesting gene mutation and accumulation of mutant protein.[12,19-21] Independent research evaluating neuroendocrine lung tumors and breast carcinomas found greater than 20% positivity to be significant both for missense mutations as well as patient prognosis.[20,21] Therefore, in this study, neoplasms that contained greater than 20% nuclear immunostaining were considered positive, and tumors that contained 20% or less immunostaining were considered negative. Overexpression of Her2/neu was observed by noting any distinct membrane staining of the tumor cells as described.[14] Hormone receptor staining was interpreted as positive if nuclear staining was observed in greater than 20% of the cells, and negative when 20% or fewer of the cells showed positive staining. Microvessel density was determined as described.[22] After staining with factor VIII, the slide was evaluated to determine the area with the highest intensity of staining. The number of vessels were counted in four 20× fields. The lowest count was discarded, and the remaining three counts averaged and expressed as the number of vessels divided by the size of the microscopic field.

Statistics

For continuous, numerical values, a *t*-test was used to compare groups. Otherwise, chi-square or Wilcoxon rank-sum test were applied.[23] Statistics were performed with the use of Statworks (Abacus Concepts, Inc., Berkeley, CA), Macintosh computer program.

RESULTS

Clinicopathologic Features of Patients With HBC and Non-HBC

Breast cancer develops at an earlier age in HBC than in non-HBC. The median age of onset was 42.4 years in BRCA1 patients ($P < .001$, versus sporadic controls), 48.4 years in BRCA2 patients ($P = .04$, versus sporadic controls), and 60.6 years in sporadic cases (Table 2). Patients with HBC have tumors of higher grade ($P < .001$ and $P = .009$ for BRCA1 cases and BRCA2 cases, respectively). The mitotic score was increased in the BRCA1 group versus the control group ($P = .003$). The BRCA2 group had more tumors with a

TABLE 2. Clinicopathologic Features of Hereditary and Nonhereditary Breast Cancer

	BRCA1	BRCA2	Non-HBC
Age			
(mean)	42.4†	48.8*	60.6
(range)	(21-63)	(34-78)	(33-87)
Tumor grade			
1	1 (4%)	4 (31%)	7 (35%)
2	7 (32%)	6 (40%)	10 (50%)
3	14 (64%)†	3 (23%)†	3 (15%)
Mitotic score			
1	5 (22%)	6 (45%)	17 (85%)
2	3 (14%)	3 (23%)	0 (0%)
3	14 (69%)†	4 (31%)	3 (15%)
Estrogen receptor			
Positive	5 (23%)	5 (38%)	16 (80%)
Negative	17 (77%)†	8 (62%)	4 (20%)
Progesterone receptor			
Positive	5 (23%)	5 (38%)	16 (80%)
Negative	17 (17%)†	8 (62%)	4 (20%)

NOTE. *P* values represent differences compared with the non-HBC group.
*$P < .05$.
†$P < .005$.

mitotic score of 3, but the difference was not statistically significant ($P = .075$). Additionally, the BRCA1 group also displayed an increase in nuclear pleomorphism versus sporadic controls ($P = .043$, data not shown). Medullary features were identified in 2 of 22 (9%) BRCA1 cases and in zero BRCA2 cases. Lobular features were seen in 2 of 22 (9%) BRCA1 cases and 1 of 13 (8%) BRCA2 cases. Tubular differentiation was seen in 2 of 22 (9%) BRCA1 cases and 3 of 13 (23%) BRCA2 cases. For both BRCA1- and BRCA2-related breast cancer, there was a decrease in estrogen and progesterone receptor positivity versus sporadic controls, but only the BRCA1 population was statistically different (Table 2).

Proliferation Markers in HBC and Non-HBC

Because of the higher mitotic scores in tumors from patients with HBC, we postulated that these neoplasms would express higher levels of the proliferation markers, topo II-alpha, and MIB1, than would tumors from patients with non-HBC. The average topo II-alpha index of 53 and MIB1 index of 57 for BRCA1 tumors is significantly higher than the topo II-alpha index of 24 and MIB1 index of 29 for the sporadic tumors (both $P < .001$, Table 3). Tumors from patients with BRCA2 mutations fall between these two values with an average topo II-alpha index of 35 and an average MIB1 index of 40. As shown in Figure 1, topo II-alpha indices correlate well with MIB1 indices in all of the breast cancers groups studied (correlation coefficient, $R = .93$).

Her2/neu, p53, and Microvessel Density in HBC and Non-HBC

Expression of Her2/neu was a relatively rare event in all of the breast cancers studied and was not statistically different between patients with HBC and non-HBC. As shown in Table 3, only one tumor with a BRCA1 mutation, one tumor with a BRCA2 mutation, and three tumors in the control population expressed this oncogene. In contrast, tumors from patients with HBC showed an increased frequency of p53 immunopositivity. Using a cutoff point of 20% as shown in Table 3, 10% of sporadic tumors were p53 immunopositive, whereas 36% of BRCA1 patients and 38% of BRCA2 patients were p53 positive ($P = .04$ and $P = .05$, respectively; chi-square analysis). If p53 positivity was compared as a continuous variable, the BRCA1 and BRCA2 groups retained statistical significance ($P = .021$ and $P = .012$, respectively) compared with the non-HBC group. However, if a cutoff point of 10% was applied, the BRCA1 group remained statistically significant ($P \leq .05$), whereas the BRCA2 group did not. The microvessel density was less in tumors from HBC patients than in tumors from non-HBC patients. The average microvessel density score was 15.5 in BRCA1 patients ($P = .03$) and 14.6 in BRCA2 patients versus 22.7 seen in patients with non-HBC.

TABLE 3. Immunohistochemical Staining Characteristics of Hereditary and Nonhereditary Breast Cancer

	BRCA1	BRCA2	Non-HBC
Topo II alpha			
(mean ± SD, %)	53 ± 26†	35 ± 22	24 ± 19
Mib1 (mean ± SD, %)	57 ± 28†	40 ± 18	29 ± 19
Her2/neu expression			
Positive	1 (5%)	1 (8%)	3 (15%)
Negative	21 (95%)	12 (92%)	17 (85%)
p53 Immunopositivity			
Positive	8 (36%)*	5 (38%)*	2 (10%)
Negative	14 (64%)	8 (62%)	18 (90%)
Microvessel density			
Microvessels/mm^2			
(mean ± SD)	15.6 ± 7.8*	14.6 ± 9.9	22.7 ± 11.8

NOTE. P values represent differences compared with the non-HBC group.
*$P \leq .05$.
†$P \leq .005$.

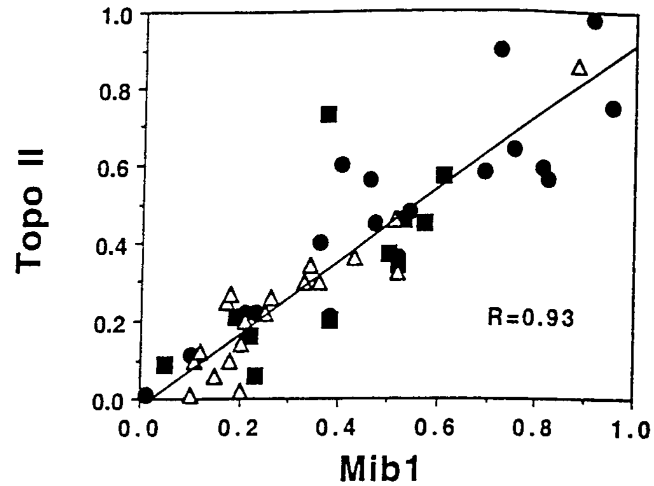

FIGURE 1. *Correlation of the topo II-alpha and MIB1 indices in HBC and non-HBC.* The topo II-alpha and MIB1 indices were determined as described in Materials and Methods. They have been divided by 100 and expressed as the fraction of positive staining tumor cells. The correlation coefficient between the topo II-alpha index and MIB1 index is 0.93 (● = BRCA1; ■ = BRCA2; △ = non-HBC).

DISCUSSION

In this work, we evaluated the pathological and clinical features of breast cancer arising in patients with HBC. Several points of caution are indicated in interpreting these results. Patients were accrued from families at high risk for HBC, and consequently do not represent a cross section of the population. The number of patients included is small; hence, the statistical power is limited. The control group was not matched for any prognostic factors such as stage, age, receptor status, or nodal status. Thus, multiple biases are possible. It was a series of sequential cases at a single hospital, and as such, it allows comparison with other series. Additionally, the use of "cutoff" values is not uniform in the literature. Both the BRCA1 and BRCA2 groups were significant compared with the non-HBC group with a cutoff point of 20%; however, the BRCA2 group lost significance with a cutoff point of 10%. The selection of a cutoff value for interpreting p53 positivity in breast carcino-

mas is critical to select differences and for evaluating prognostic significance.[20]

In this study, the BRCA1 cases or the BRCA2 cases were significantly different compared with the nonmatched control group in terms of age, tumor grade, mitotic score, ER positivity, PR positivity, topo II-alpha staining, Mib1 statining, p53 immunopositivity, and microvessel density (Tables 2, 3). The only significant difference between the BRCA1 and BRCA2 groups was found for tumor grade ($P < .05$) with high-grade tumors observed for 64% of BRCA1 cases and 23% of BRCA2 cases (Table 2).

In confirmation of previous data, we found that the age of onset in patients with HBC is roughly a decade earlier than in patients with non-HBC.[7,9] The frequency of medullary and lobular features in BRCA1- and BRCA2-related breast cancer observed here is consistent with previous reports.[7,10] In addition, patients with HBC generally have neoplasms that show adverse histological features. These include tumors with high nuclear grade, high proliferation indices, lack of hormone receptor positivity, and an increase incidence of p53 immunopositivity.[6,7,10,12,13] In spite of these negative prognostic markers, other investigators have shown that patients with HBC have comparable or improved survival compared with patients with non-HBC.[6-9,24,25] In a larger study that included 30 BRCA1 patients and 20 BRCA2 patients, we evaluated overall survival compared with sporadic controls matched for tumor size, age, and date of diagnosis, and there were no differences in survival at 5 or 10 years.[24] Thus, our data suggest that survival is similar for BRCA1 patients, BRCA2 patients, and non-HBC patients.

In one report of patients with BRCA1-related breast cancers, grade was believed to segregate as a genetic trait within families. Moreover, this was attributed to mitotic index segregation, and a possible genotype-phenotype correlation was suggested. Although our patient numbers are small, especially when evaluated per kindred (Table 1), our data do not confirm this hypothesis. A normal range was observed for all evaluated parameters within families, including grade, mitotic index, Mib1, topo II-alpha, and p53.

To understand this apparent paradox between histological findings and clinical course, we investigated the expression of several markers, which have not previously been evaluated in HBC. Amplification of HER2/neu oncogene has been correlated with more aggressive disease. The number of cases in our study that showed increased expression of this oncogene was too small to yield statistically significant results.

Microvessel density has also been suggested to yield prognostic information in breast cancer. Low microvessel density suggests a more favorable clinical course.[15,22] Interestingly, we found that the average microvessel density score in tumors from patients with BRCA1-related breast cancer was statistically lower than that observed in a control group. The prognostic implications of microvessel density and its reproducibility is controversial. It is possible that tumors arising in patients with HBC may have a decreased ability to undergo angiogenesis compared with non-HBC tumors, and this may modify the clinical course. Further studies will be required to explore this observation.

Our proliferation data suggest another possible molecular mechanism that may partially explain the clinical response of patients with HBC to therapy. It has been suggested previously that breast cancers with a high population of cycling cells have a high likelihood of responding to chemotherapy.[26] However, those tumors that do not initially respond or in which a large number of cells are not killed, would show an early relapse.[26] Thus, the proliferation index of a breast cancer could be viewed as showing both positive and negative clinical correlations. We found, as others also have, that tumors from patients with HBC have higher proliferation indices than tumors from patients with non-HBC.[7,8,10] In addition, we have shown in this study that these high proliferation indices correlate with increased expression of topo II-alpha. Topo II-alpha is an enzyme elevated in proliferating cells, where its function is to separate intertwined DNA strands before mitosis. Although clearly a marker of cell proliferation, topo II-alpha is also the molecular target of many clinically used antitumor drugs.[14] Cells that express high topo II-alpha levels are drug sensitive, and cells that express low topo II-alpha are drug resistant. Some of the drugs that target topo II-alpha such as doxorubicin are used in the treatment of breast cancer. It is possible that the increased expression of topo II-alpha in HBC might play an important role in the relatively favorable clinical response of these patients to chemotherapy. If resistant clones do not arise early in the course of HBC, then the high proliferative indices in HBC tumors could have positive prognostic implications. The decreased level of angiogenesis in BRCA1-related tumors may reduce the rate of early metastatic spread of tumor cells. The positive prognostic implications of high tumor cell proliferation and decreased angiogenesis might balance out negative indicators found in this group of tumors such as increased frequency of p53 immunopositivity, high nuclear grade, and lack of hormone receptor positivity. Thus, it is plausible mechanistically that patients with HBC may have a similar clinical outcome to patients with non-HBC. Further work correlating therapy and clinical outcome with molecular markers in HBC and non-HBC would be useful to answer this question.

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Ethnic Differences in Cancer Risk Resulting from Genetic Variation

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Ethnic differences in cancer incidence and mortality exist and are probably the result of genetic and epidemiological risk factors. Genetic differences caused by founder mutations are reviewed, with special emphasis on mutations in *BRCA1* and *BRCA2*. Germline mutations in cancer susceptibility genes have been identified in individuals of all races and ethnic groups. Differences among ethnic groups for cancer risks have been recognized, and a proportion of the differences may be the result of founder mutations within these genes. The *BRCA2* 999del5 mutation in Iceland and the three *BRCA1* and *BRCA2* mutations in Ashkenazic Jews have been well characterized and were easy to study because the patient population and anonymous samples were readily available and ethnicity was known. Mutations in *BRCA1* and *BRCA2* probably account for approximately 3 to 10% of breast cancer in the general population and a much higher proportion in those with a strong family history of breast and ovarian cancers and in those of Ashkenazic Jewish descent. However, no overall increased risk of breast or ovarian cancers exists among Ashkenazic Jewish women compared with non-Jewish Caucasians. Some ethnic variation in cancer risk may be explained by founder mutations identified in cancer-predisposing genes. Knowledge acquired by studying the effect of a single mutation in a well defined population may be applied to larger, more heterogeneous populations. Individuals from all racial and ethnic groups carry deleterious mutations. Mutations are simply easier to find and characterize when identified in a specific ethnic group. ***Cancer*** **1999;86:2575–82.**

KEYWORDS: genetic variation, germline mutations, *BRCA1*, *BRCA2*, cancer risk estimates

Cancer is caused by both exogenous and endogenous factors. The published probabilities of developing cancer are averages across the population. They do not factor in individual behavior and risk factors. Demographic factors include age, sex, race, socioeconomic status, and geographic location.[1] With increasing age, there is an increased risk for many cancers, including breast and prostate cancers. Sex is a risk factor for some cancers because some are sex-limited (e.g., ovarian and prostate cancers), and others are more common in one sex; e.g., breast cancer is 100 times more common in women than in men.

Other risk factors include exposure to physical and biologic agents (chemical exposures, drugs, infectious agents,and so forth), which may increase risks of certain cancers such as lung and gastric cancers.[1] Lifestyle factors, including alcohol use, smoking, diet, and exercise, may also affect cancer risk.[1] For breast cancer, known reproductive factors such as age at menarche and menopause, age at

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TABLE 1
Examples of Founder Mutations Identified in *BRCA1* and *BRCA2*

Population	Mutations	References
Ashkenazic Jews	*BRCA1*-185delAG, 5382insC; *BRCA2*-6174delT	Struewing et al.,[36] Tonin et al.,[37] Neuhausen et al.[38]
Icelanders	*BRCA2*-999del5	Thorlacius et al.[28]
Dutch	*BRCA1*-2804delAA, del510, del3835; *BRCA2*-5573insA	Peelen et al.,[31] Petrij-Bosch et al.[32]
Norwegians	*BRCA1*-1136insA	Andersen et al.[29]
Swedes	*BRCA1*-Q563X, 3166ins5, 1201del1, 2594delC; *BRCA2*-4486delG	Johannsson et al.,[27] Hakansson et al.[39]
African Americans	*BRCA1*-M1775R, 1832del5, 5296del4	Gao et al.[35]

first pregnancy, number of full-term pregnancies, and oral contraceptive use are important.[2,3] One of the largest risk factors is a family history of cancer. Relative risks range from 2 to 9 depending on the type of cancer, age, and number of first-degree relatives affected by the disease.[1] Segregation analyses of pedigrees often suggest a genetic basis for the family history.

Ethnic Differences in Cancer Rates

Ethnic differences in cancer incidence and mortality are well documented.[4] For example, African-American men have the highest incidence of prostate cancer and Japanese men living in Japan have the lowest incidence.[5–7] With migration to the United States, the rate of prostate cancer increases in Asians,[8] suggesting that diet or lifestyle factors contribute to development of the disease.[9] It also has been hypothesized that a portion of the observed ethnic differences in cancer susceptibility may be explained by genetic factors from mutations in rare genes that confer high risk[10,11] and/or from alleles of specific genes that confer modestly increased risk, such as androgen metabolism genes.[12,13] Clear ethnic differences have also been observed in breast cancer populations. Hispanic and Native American women have the lowest incidence of breast cancer compared with non-Hispanic Caucasians and African Americans.[4] Hypotheses regarding lifestyle, reproductive, and screening factors explain some of the differences in breast cancer incidence.[14] However, the ways that different risk factors specifically act and interact to promote cancer are largely unknown.

An endogenous factor that must be considered is the role of inherited (germline) mutations in ethnic differences in cancer risk. A genetic predisposition probably accounts for approximately 5 to 10% of cancer. Genes for more than 20 cancer syndromes have been identified. Differences among ethnic groups for cancer risks in some of these genes have been recognized and are caused by a common germline mutation within an ethnic group.

Ethnic differences may arise from founder effects, which occur when a population is established by a small number of people. Once the population expands, the mutation in one of the founders then becomes prevalent in a larger proportion of the population. The evolutionary significance of founder effects can be studied by following pedigrees for many generations and examining genetic relationships. Examples of populations in which founder effects are well documented include Afrikaners of South Africa,[15] Finns,[16] Ashkenazic Jews,[17] and French Canadians.[18] Examples specific to cancer genes are a founder mutation in *APC* found in Ashkenazic Jews,[19] one in *hMLH1* found in Finns,[20] one in *VHL* found in Germans,[21] one in *CDKN2* found in Dutch,[22] and mutations found in *BRCA1* and *BRCA2* in many different groups.[23] This review focuses on founder mutations identified in *BRCA1* and *BRCA2*, two genes that predispose individuals primarily to breast and ovarian cancers.

Founder Mutations in *BRCA1* and *BRCA2*

For *BRCA1* and *BRCA2*, more than 300 mutations have been identified in individuals of all racial and ethnic groups.[24,25] As DNA from individuals is evaluated, recurring mutations are identified. These are then further examined to determine if they are founder mutations (e.g., a shared haplotype) or ones that arose two or more times by chance. Founder mutations for *BRCA1* and *BRCA2* have been described in French Canadians,[26] Swedes,[27] Icelanders,[28] Norwegians,[29] Finns,[30] Dutch,[31,32] Russians,[33] Japanese,[34] African Americans,[35] and Ashkenazic Jews.[36–38] A partial list of mutations is presented in Table 1.

Complex and controversial issues that arise from genetic research pertain to who should be offered predictive testing and when it should be done. An important consideration for testing is the probability that an individual with breast or ovarian cancer (or both) will have a mutation in *BRCA1* or *BRCA2*. Estimates are that the gene frequency of a major gene(s) for breast cancer is 0.0033[40] and of *BRCA1* is 0.006,[41] so

TABLE 2
Prevalence of the Icelandic *BRCA2* Founder Mutation 999del5

Group	No.	No. of occurrences	Comments	References
Breast cancer population-based	520	3 (0.6%)		Thorlacius et al.[42]
Families	21	16 (76.0%)	9/16 had male breast cancer	Thorlacius et al.[28]
Male breast cancer cases	30	12 (40.0%)	In the 9 families	Thorlacius et al.[28]
Female breast cancer cases	632	49 (7.7%)		Thorlacius et al.[42]
Prostate cancer population-based	65	2 (3.1%)	Significantly worse survival	Sigurdsson et al.[43]

TABLE 3
Frequency of *BRCA1* and *BRCA2* Mutations in Ashkenazic Jews

Source	No.	185delAG	5382insC	6174delT	References
Population-based	varied	0.8–1.1%	0.13–0.3%	0.9–1.5%	Struewing et al.,[36] Roa et al.,[45] Oddux et al.[46]
BC < age 42	80	20.0%	4.0%	8.0%	Neuhausen et al.,[38] Offit et al.[47]
BC 42–50 yrs	27	30.0%	4.0%	7.0%	Neuhausen et al.,[38] Offit et al.[47]
BC only families	138	20.0%	5.0%	4.0%	Tonin et al.[48]
B/O families	82	52.0%	16.0%	5.0%	Tonin et al.[48]

BC: breast cancer; B/O: breast and ovarian cancers.
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that the likelihood of an individual carrying a mutation is low. Many studies have been performed to identify mutation prevalence and to develop probability models to predict a mutation carrier before testing. Much more information is available for the *BRCA2* 999del5 mutation in Icelanders and the three founder Ashkenazic Jewish mutations because a large number of samples are available. In addition, mutation detection is rapid and inexpensive compared with screening entire genes.

The population prevalence and proportion of individuals with breast, ovarian, and prostate cancer with the *BRCA2* 999del5 mutation in the Icelandic population are shown in Table 2. This mutation in Iceland is approximately 20 times more prevalent (0.6%)[42] than the estimated allele frequency in the general population.[40] In Icelandic breast cancer cases unselected for a family history, it accounts for 7.7% of female breast cancer diagnosed at any age and for 24% of those diagnosed in women younger than 40 years.[42] It also was the cause of disease in the majority (76%) of high-risk breast cancer families studied.[28] For males, it accounts for 40% of male breast cancer and 3.1% of prostate cancer.[42, 43] The risk ratio of prostate cancer in first-degree relatives of mutation carriers is 4.6.[43] This mutation with the same haplotype has also been seen in Finland.[30,44]

Table 3 is a similar table for the three common mutations identified in Ashkenazi Jewish breast and ovarian cancer patients. The population prevalence for these three mutations combined is 2 to 2.5%,[36,45,46] which is approximately 10 to 50 times higher than the allele frequency in the general population. Based on a number of studies, approximately 30% of breast cancer diagnosed in those younger than 40 years and 39% of ovarian cancer diagnosed in those younger than 50 years in this population are caused by one of the three founder mutations.[49–51] Therefore, even in the absence of a strong family history, Ashkenazic Jewish women with breast or ovarian cancers have a much higher probability than do non-Jewish women of being *BRCA1* or *BRCA2* mutation carriers. However, even though mutations in these genes are more common in Ashkenazic Jewish women, there is little to no overall increased risk of breast or ovarian cancers among these women compared with non-Jewish Caucasians.[52] Egan et al.[52] reported a suggestion of an increased risk of breast cancer in Jewish women with a family history, which could reflect the frequency of the founder *BRCA1* and *BRCA2* mutations.

In general, mutations in both *BRCA1* and *BRCA2* in one individual are rare, given the frequency of mutations. In the Ashkenazic Jewish population, the *BRCA1* 185delAG and *BRCA2* 6174delT both occur with frequencies of 1%, so it is not surprising that Jewish women with both *BRCA1* and *BRCA2* mutations have been identified[53,54] (Neuhausen, unpublished data). Although these women are carrying two deleterious

mutations, age of onset of cancer and prognosis do not appear to be different than in those with only one mutation.

The focus on the "Jewish" mutations has caused concern in the Jewish community that *BRCA1* and *BRCA2* are peculiar to the Jewish people.[55,56] But that is not the case. *BRCA1* and *BRCA2* mutations have been identified in individuals of all racial and ethnic groups. As referenced above, relatively homogenous populations have founder mutations in which small genetic alterations that cause disease are easy to find. These groups (e.g., Ashkenazic Jews, French Canadians, Finns, Afrikaners) are then the first to be studied because information obtained from studying the effect of a mutation in a well defined population may be beneficial for determining effects in larger, more heterogeneous populations. The *BRCA1* and *BRCA2* mutations in Ashkenazic Jewish populations were easy to study because the patient population and anonymous samples from prenatal testing were readily available and identified as being of Jewish ancestry. Ashkenazic Jews do not have more defective DNA than any other ethnic group does, and they do not have higher rates of hereditary diseases than others. The same is true for other ethnic groups in which founder mutations have been identified.

Frequency of *BRCA1* and *BRCA2* Mutations

It is estimated that in the general population, approximately 6 to 7% of breast cancer cases and 10% of ovarian cancer cases averaged across all ages of onset result from mutations in breast cancer susceptibility genes.[57] The frequency of *BRCA1* and *BRCA2* mutation carriers in women with breast or ovarian cancer (or both) depends on the study population.

In a large clinic-based study, the minimum criterion for entry was breast cancer at younger than 50 years or ovarian cancer at any age and a minimum of one affected first-degree or second-degree relative with breast cancer younger than 50 years or ovarian cancer at any age.[58] Mutations in *BRCA1* or *BRCA2* were detected in 45% (50 of 101) of women with at least two affected relatives and in 22% (20 of 89) of women with only one affected relative.[58] In non-Jewish women with breast or ovarian cancer (or both)and a family history of breast and/or ovarian cancer, the risk of carrying a mutation in *BRCA1* and *BRCA2* was approximately the same as in Jewish women (38.7% and 42.6%, respectively).[58] The presence of a strong family history of disease was a significant predictor of the likelihood of carrying a *BRCA1* or *BRCA2* mutation.

The results from this cohort of breast and/or ovarian cancer cases with a strong family history can be compared with those of other clinic-based studies and those of population-based studies. In two clinic-based studies that selected women based exclusively on age of onset as a predictor of *BRCA1* status, 8% and 10% of women younger than 35 and 30 years, respectively, were found to carry germline mutations in the *BRCA1* gene.[59,60] In a population-based study, Malone et al.[61] reported that of 208 Caucasian women diagnosed with breast cancer before their 45th birthdays who had a family history of breast cancer in first-degree relatives, 15 (7.2%) had germline mutations in *BRCA1*. In this study, the younger the age at diagnosis of cancer and the stronger the family history, the higher the percentage of mutations found. In another population-based study, Newman et al.[62] reported that *BRCA1* mutations were found in only 3.3% (4 of 120) of Caucasian women with breast cancer diagnosed between ages 20 and 74 years. Family history was the greatest predictor of *BRCA1* mutation status, based on both number of affected relatives and presence of ovarian cancer in a relative.[62] The conclusion from these studies is that the stronger the family history of breast and/or ovarian cancer and to a lesser extent, the younger the age at diagnosis, the more likely a breast or ovarian cancer case is to carry a mutation in *BRCA1* or *BRCA2*.

Most breast cancer studies have examined women of Northern European ancestry. African American women, who have a higher incidence of early onset breast cancer,[4] have yet to be studied extensively. One can infer from the available data for *BRCA1* that mutations in African American differ from those in Caucasians and that there also may be founder effects in this population. Three novel *BRCA1* mutations were identified in five of nine (56%) African-American families screened for mutations.[35] In the population-based study of Newman et al.,[62] no mutations were identified in 99 African-American women with breast cancer. This suggests that, as in Caucasians, the incidence of *BRCA1* mutations in African Americans is most likely to occur in patients with a strong family history of breast cancer and a young age at diagnosis.

Models have been developed to predict the likelihood that a woman has a germline *BRCA1* or *BRCA2* mutation.[58,63–66] In two separate studies, researchers at the University of Pennsylvania (Philadelphia, PA)[63] and at Myriad Genetic Laboratories (Salt Lake City, UT)[64] screened for mutations in *BRCA1* then used logistic regression analysis to develop models to evaluate the probability of a woman carrying a deleterious mutation. For the model developed by Couch et al.,[63] the predicted probability is the same for a woman with breast or ovarian cancer and for her family. Regression variables included age at diagnosis, family history of breast and ovarian cancer, both breast and ovarian cancer in a single family member, and Ashkenazic

TABLE 4
Estimated Cumulative Risks of Developing Breast and Ovarian Cancers

Breast cancer by age	BCLC-*BRCA1*[68]	BCLC-*BRCA2*[69]	Ashkenazi-*BRCA1/BRCA2*[70]	Population w/$BRCA_n$[40]	General population[1]
30	0.036 (0–0.14)	0.006 (0–0.019)	0	0.017	0.0002
40	0.18 (0–0.35)	0.12 (0–0.24)	0.15 (0.07–0.23)	0.144	0.005
50	0.49 (0.28–0.64)	0.28 (0.09–0.44)	0.33 (0.23–0.44)	0.376	0.01
60	0.64 (0.43–0.77)	0.48 (0.22–0.65)	0.54 (0.38–0.68)	0.548	0.02
70	0.71 (0.53–0.82)	0.84 (0.43–0.95)	0.56 (0.40–0.73)	0.647	0.04
Ovarian	0.42	0.27 (0–0.47)	0.16 (0.06–0.28)	0.10	0.01

BCLC: Breast Cancer Linkage Consortium; $BRCA_n$: any breast cancer susceptibility gene including those not yet identified.

Jewish descent. The model developed by Shattuck-Eidens et al.[64] included the above variables as well as the type of cancer and number of affected relatives. In a recent analysis, Frank et al.[58] calculated the probability that a woman with breast and/or ovarian cancer who has a strong family history of breast and ovarian cancer is carrying a *BRCA1* or *BRCA2* mutation. In this cohort, Ashkenazic Jewish status was not included in the predictive model, because the Ashkenazic Jewish group did not have a significantly different percentage of mutations compared with the non-Jewish group. These results suggest that a strong family history is a powerful predictor of the likelihood of carrying a mutation, regardless of ethnicity.[58] Researchers at Duke University[65,66] developed a model to evaluate the probability that a woman carries a mutation in *BRCA1* or *BRCA2*, based on her family history of breast and ovarian cancers. Using a Bayesian approach, the Duke researchers incorporate information about the family's possible genetic status, age-specific incidence of breast and ovarian cancers in carriers, and mutation prevalence in the population. These values can be changed to customize the model for subpopulations. For example, for Ashkenazic Jewish women, different allele frequency and age-specific penetrance are used in the calculation to obtain more accurate estimates for use in counseling.

Likelihood of a Mutation Carrier Developing Cancer

Determining the probability that an individual is carrying a *BRCA1* or *BRCA2* mutation is only half of risk assessment. The other probability that must be determined is the likelihood of a mutation carrier developing cancer by a given age (i.e., age-specific penetrance). This is the point at which risk assessment becomes especially problematic, because all the factors that contribute to the development of cancer have not been identified. Not all individuals who carry mutations develop breast cancer or any other cancer. Expression is variable. For example, *BRCA2* mutation carriers may develop breast cancer, ovarian cancer, pancreatic cancer, fallopian tube cancer, or ocular melanoma. Even among families with founder mutations, there appear to be differences in age of onset of cancer and in the type of cancers that develop.[26,28,37,67] Expression and penetrance can vary from early onset bilateral breast cancer with ovarian cancer to late-onset breast cancer and from no other cancers in the family to additional cancers such as prostate, pancreatic, and other cancers. Therefore, it is not possible to assign mutation-specific risks. However, it is important to provide individuals with estimates of the likelihood of developing cancer.

The risk of developing breast or ovarian cancer when carrying a mutation varies in relation to the cohort studied (Table 4). The Breast Cancer Linkage Consortium (BCLC) risk estimates[68,69] are derived from families with several affected breast and or ovarian cancer cases. The estimates of Struewing et al.[70], which are for Ashkenazic Jews with any of three founder mutations, appear to be lower than the BCLC estimates. However, the estimates are not inconsistent, given that the confidence intervals overlap and both have similar risks (55%) by age 60. The existence of true differences could be explained by ascertainment- or mutation-specific differences. The Claus estimates[40] are for individuals in the general population who carry a susceptibility allele ($q = 0.0033$). The risks for developing breast or ovarian cancer are high for mutation carriers, regardless of the variation in the estimates. They may be lower in those mutation carriers with little or no family history. The general population rates are also shown,[1] and they are relatively low. Estimates are that 1 in 8 women in the United States will develop breast cancer over the course of a lifetime, which includes the 5 to 10% of women carrying a high-

penetrance gene predisposing to breast and/or ovarian cancers.

BRCA1 and *BRCA2* mutations are certainly important determinants of risk for breast and/or ovarian cancers, but they are not the only ones. Many women who have a family history of breast and/or ovarian cancer and do not have a *BRCA1* or *BRCA2* mutation may have a mutation in undiscovered genes. Moreover, some women may be *BRCA1* or *BRCA2* mutation carriers in the absence of a strong family history. This is especially true in women of Ashkenazic Jewish descent. For a subset of women, better predictions about their likelihood of developing breast and/or ovarian cancer at an early age can be made using *BRCA1* and *BRCA2* test results. However, even knowing mutation status does not always allow for valid risk estimates. Missense mutations are a good case in point, because the role of most of them is unknown.

CONCLUSION

One conclusion that can be drawn from this area of research is that there is ethnic variation in cancer risk that is probably the result of both genetic and epidemiological factors. Many genes have been isolated that are known to predispose humans to cancer. Founder mutations have been identified in many of these genes in different ethnic groups. Their further characterization is important because it will allow for more accurate risk assessment and more astute genetic counseling. However, the presence or absence of a founder mutation does not exclude the possibility of another mutation.

The risk of breast and ovarian cancers in mutation carriers is much higher than that in the general population, even given variable estimates depending on the population studied. Although estimates for risks of developing other cancers are not generally available, genetic counselors and physicians must be aware of the possibility of increased risks for other cancers as well.

Knowledge of which factors—genetic, environmental, or both—affect cancer development is essential for designing effective screening methods, providing information on ways to reduce cancer risk, and developing effective treatments once cancer develops. By studying the effect of a single, frequent mutation (founder mutation) in a well defined population, knowledge is gained that can be applied to larger, more heterogeneous populations. The founder mutations in *BRCA1* and *BRCA2* in Ashkenazic Jewish populations are the first to be examined in detail, and the data that are generated as a result of these studies are likely to provide information that will aid in the development of strategies for more successful prevention and treatment of breast and ovarian cancers.

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Commentary

Founder populations and their uses for breast cancer genetics

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Abstract

Numerous founder mutations have been reported in *BRCA1* and *BRCA2*. For genetic screening of a population with a founder mutation, testing can be targeted to the mutation, allowing for a more rapid and less expensive test. In addition, more precise estimates of the prior probability of carrying a mutation and of the likelihood of a mutation carrier developing cancer should be possible. For a given founder mutation a large number of carriers are available, so that focused scientific studies of penetrance, expression, and genetic and environmental modifiers of risk can be performed. Finally, founder populations may be a powerful resource to localize additional breast cancer susceptibility loci, because of the reduction in locus heterogeneity.

Keywords: *BRCA1*, *BRCA2*, breast cancer genes, founder mutations, genetic epidemiology

Introduction

Ethnic differences in the prevalences of many diseases have been observed. For example, sickle-cell anemia in individuals of African descent, Tay–Sachs disease in Ashkenazi Jews [1], and approximately 30 diseases in Finland [2] are more prevalent than in other populations. A likely reason for a preponderance of a disease in a specific population is a founder effect. Founder effects occur when a population is established by a small number of people or when a bottleneck occurs that reduces the population to a small number. When population expansion occurs, the mutation in a founder becomes prevalent in a larger proportion of the population. There may also be a selective advantage to the mutation carrier. By following genetic relationships over many generations, the significance of founder effects can be studied. Diamond and Rotter [3] reviewed studies of the Afrikaner population of South Africa. In 1652, one founding immigrant carried a gene for Huntington's chorea and one brother–sister pair carried a gene for lipoid proteinosis. The result of founder effects is that these diseases are more common in South Africa than in Holland from where the carriers emigrated.

Founder populations can be useful in genetic studies, particularly for genetic mapping of complex traits. There is little genetic heterogeneity, so that the majority of individuals with disease will carry the same gene mutation. Linkage disequilibrium between the site of the gene and close markers will exist, so that shared regions of the genome cosegregating with disease can be more readily discerned. As an example, Hirschprung's disease has been described in individuals of many different backgrounds. Using a Mennonite population, in which all affected individuals could be traced to a single common ancestral couple, one of the genes for the disease was localized and subsequently identified [4].

Once founder mutations are identified, researchers are able to examine prevalence of mutations in different populations and mutation-specific effects on penetrance and

disease phenotype. Possibly, better estimates of risk for individuals in populations with founder mutations can be calculated. This editorial focuses on founder populations in genetic studies of breast cancer.

Prevalence of mutations in *BRCA1* and *BRCA2*

BRCA1 and *BRCA2*. two genes predisposing to breast and ovarian cancers, were isolated in 1994 and 1995, respectively [5,6]. Since that time, researchers have been screening for mutations in high-risk breast and/or ovarian cancer families and in population-based samples of women with these cancers to determine the prevalence and range of mutations. Over 1300 distinct variants have been found across all population groups, of which approximately 700 are identified as causal [7,8]. A number of these mutations have been identified multiple times [8]. Many of these common mutations have been classified as founder mutations on the basis of a shared haplotype in the genomic region containing the gene. Founder mutations for *BRCA1* and *BRCA2* have been described in numerous populations (Table 1), as well as across populations. For example, *BRCA1* 5382insC has been reported in individuals of Jewish, Dutch, Lithuanian, Russian, Hungarian, Germanic, French, Italian, British, and French–Canadian ancestry [8]. This suggests that this is a relatively old mutation that has spread through migration.

Relative ages of several founder mutations have been investigated by examining the distance over which haplotypes are conserved [9,10]. Based on the general age of a mutation and historic data on migration and social patterns, the origin and subsequent migration of specific mutations may be described. Now that a large number of mutation carriers have been identified the Breast Cancer Linkage Consortium is undertaking such a study for a set of founder mutations.

Assessment of risk

Genetic screening

Since the isolation of *BRCA1* and *BRCA2*, genetic testing for mutations is becoming more common in clinical genetic practice. Important considerations are who should be offered predictive testing and when it should be done. In general, mutations in *BRCA1* and *BRCA2* are rare, probably accounting for less than 5% of breast cancers and 10% of ovarian cancers in the population [11,12]. The frequency of *BRCA1* and *BRCA2* mutation carriers in women with breast and/or ovarian cancer is dependent on the study population, and is highest in young women with breast cancer who have a strong family history of breast and/or ovarian cancers. An essential issue for testing is the probability that an individual, with breast or ovarian cancer or with a family history of cancer, will carry a mutation in *BRCA1* or *BRCA2*. Probability models have been developed to predict the likelihood of being a mutation carrier before testing [13–16]. Prior probabilities vary depending on the model used.

Table 1

Examples of *BRCA1* and *BRCA2* founder mutations

Population	Mutation	Reference
African–Americans	*BRCA1* 943ins10	[40,41]
	BRCA1 M1775R	
Ashkenazi Jews	*BRCA1* 185delAG *BRCA1* 5382insC *BRCA2* 6174delT	[31,34,38]
Belgians	*BRCA1* IVS5 +3A>G	[42]
Dutch	*BRCA1* 2804delAA	[17,43]
	BRCA1 IVS 21-36del510	
	BRCA1 IVS 12-1643 del3835	
	BRCA2 5573insA	
Finns	*BRCA1* 3745delT	[27]
	BRCA1 IVS 11-2 A>G	
	BRCA2 999del5	
	BRCA2 IVS23-2A>G	
French–Canadians	*BRCA1* R1443X *BRCA2* 8765delAG	[39,44]
Germans	*BRCA1* 5382insC	[45]
	BRCA1 C61G	
Icelanders	*BRCA2* 999del5	[28]
Latvians	*BRCA1* C61G	[46]
	BRCA1 5382insC	
	BRCA1 4153delA	
Norwegians	*BRCA1* 1675delA	[47–49]
	BRCA1 1135insA	
Russians	*BRCA1* 5382insC	[50]
	BRCA1 4153delA	
Swedes	*BRCA1* Q563X	[51]
	BRCA1 3166ins5	
	BRCA1 1201del11	
	BRCA1 2594delC	
	BRCA2 4486delG	

For genetic testing, there are several advantages to knowing the founder mutation(s) in a population. First, a more accurate estimate of the prior probability of carrying a mutation should be possible. Second, for mutation detection, testing can be targeted to the founder mutation, allowing for a more rapid and less expensive test. Third, most of the mutation detection techniques are unable to detect large deletions and insertions, so that these types of mutations, which may account for 5–15% of deleterious mutations, would be undetected. If one of these mutations is

known in the population, however, a technique that detects it can be used for mutation screening. For instance, there are two large deletion founder mutations in the Dutch that would not be detectable with standard techniques [17].

Age-specific penetrance

Once an unaffected mutation carrier is identified, the question becomes what is the likelihood that she will develop cancer by a given age (age-specific penetrance). It is especially difficult to answer, because not all factors that contribute to the development of cancer are known. A proportion of individuals who carry mutations will not develop breast cancer or any other cancer. On the basis of estimates from population-based studies of women aged 40 years or younger to estimates from high-incidence breast cancer families of Northern European descent, the cumulative risk of breast cancer by age 70 years for *BRCA1* and *BRCA2* mutation carriers is between 40 and 80% [18–20]. Mutation-specific differences may also be important. There are regions in *BRCA1* and *BRCA2* in which mutations confer higher risks for developing ovarian cancer: 5′ of codon 1435 in exon 13 of *BRCA1* [21] and a 3.3 kilobase region of exon 11 in *BRCA2* (denoted the Ovarian Cancer Cluster Region) [22]. It is unclear whether the differences in risk for ovarian cancer are due to a difference in penetrance of the mutations for breast cancer or ovarian cancer, or both. For *BRCA2*, it has been suggested that the breast cancer risk remains the same, but that the ovarian cancer risk increases [20]. Expression is also variable [23]. In a population with a defined founder mutation(s), more accurate assessment of the likelihood of developing cancer for a mutation carrier should be possible.

Founder mutations

BRCA1 and *BRCA2*

An example of a recurrent, founder mutation is the *BRCA2* 999del5 mutation in the Icelandic population. No other *BRCA2* mutations have been reported in this population. The 999del5 is approximately 20 times more prevalent (0.6%) [24] than the estimated allele frequency of *BRCA2* in the general worldwide Caucasian population [25]. This mutation with the same haplotype was also found in Finland [26,27]. In Iceland, it was the cause of female breast cancer in the majority (76%) of 21 high-risk breast cancer families studied [28]. In nine of those 16 families, male breast cancer was also present [28]. In 632 Icelandic breast cancer cases unselected for a family history, 7.7% of female breast cancer diagnosed at any age and 24% of those diagnosed at age 40 years or younger carried the *BRCA2* 999del5 mutation [24]. This mutation is also responsible for a proportion of prostate cancer, as it accounted for 3.1% (in two out of 65 individuals) of prostate cancer cases in a population-based series of cases [29]. Because this is the only *BRCA2* mutation found in Iceland, genetic testing can be targeted to this mutation. Second, because there are a large number of individuals, both symptomatic and asymptomatic, who carry this mutation, it may be possible to develop more accurate risk estimates for mutation carriers. Age-specific penetrance has been calculated to be 17% by age 50 years and 37.2% by age 70 years [30]. This is a lower frequency than that reported in other studies of *BRCA1* and *BRCA2* penetrance.

Three founder mutations have been observed in Ashkenazi Jewish breast and ovarian cancer patients. The *BRCA2* 6174delT mutation has been seen only in Ashkenazi Jews [31], with a frequency of 0.9–1.5% [32,33]. The founder *BRCA1* 185delAG mutation, with a frequency of 0.8–1.1% in Ashkenazi Jews [32,34], is also observed in Sephardic Jews, indicating an older origin. The 185delAG mutation has also been observed in individuals of English origin but on a different haplotype, which suggests a different origin. The third founder mutation, *BRCA1* 5382insC, has a frequency of 0.13–0.3% in Ashkenazi Jews. The 5382insC mutation is observed in many populations, and the vast majority of carriers share the same core haplotype (Szabo C, personal communication). The population prevalences for these three mutations combined is 2–2.5% [32–34], which is approximately 10–50 times higher than the allele frequency in the general population. Few other *BRCA1* or *BRCA2* mutations have been identified in Jewish breast or ovarian cancer cases. In this population, approximately 30% of breast cancers diagnosed at less than 40 years of age and 39% of ovarian cancers diagnosed at less than 50 years of age are caused by these mutations [35,36]. Thus, Ashkenazi Jewish women with breast or ovarian cancers have a much higher probability than non-Jewish women of being *BRCA1* or *BRCA2* mutation carriers. Because these mutations are so common in Ashkenazi Jewish women, they are commonly tested as a panel, regardless of whether a mutation has already been identified in a family member. A woman may carry a second mutation not present in the first family member tested and, by testing the panel, it is detected. Without knowledge of the founder mutations, a false-negative test result for an individual with a mutation-specific test could result.

Even among families with founder mutations, there appear to be differences in age of onset of cancer and in the type of cancers that develop [28,37–39]. This suggests that there are both genetic and lifestyle factors that modify penetrance of *BRCA1* and *BRCA2*. By studying a cohort of individuals with the same mutation, one may be able to distinguish factors that affecting penetrance, because there will not be a confounding effect from genotype–phenotype correlations from location of the *BRCA1*/*BRCA2* mutation in the individual. Once a risk factor is identified in one subgroup of mutation carriers it would need to be tested across other mutation carriers. Subsequently, it

would need to be tested in a population-based case–control study, in order to determine how important the risk factor is in the general population.

Other genes

BRCA1 and *BRCA2* mutations are certainly important determinants of risk for breast and/or ovarian cancers, but they are not the only ones. Many women, who have a family history of breast and/or ovarian cancer and do not have a *BRCA1* or *BRCA2* mutation, may have a mutation in undiscovered genes. After accounting for *BRCA1* and *BRCA2*, Peto *et al* [12] suggested that there are several other genes, possibly of lower risk, that account for a proportion of breast cancers. This complexity makes localizing additional genes problematic. Studying families identified from populations in which there are likely to be founder mutations may be extremely useful for localizing additional genes. For example, in Iceland researchers may have been able to localize *BRCA2* by studying male breast cancer cases from high-risk families and looking for regions of the genome with excess sharing. Researchers have suggested studying high-risk Ashkenazi Jewish breast cancer families that do not have a *BRCA1* or *BRCA2* mutation in order to localize *BRCA3*. Localization will be promoted by minimizing the effects of genetic heterogeneity.

Conclusion

Founder mutations allow for focused scientific studies of penetrance, expression, and genetic and environmental modifiers of risk. The results from these studies may be very useful for understanding the role that these genes play in the incidence of breast cancer in order to target genetic testing, to provide individual risk assessment, and to design better therapeutic strategies. Localization studies to find *BRCA3*, using founder populations, may be more successful than traditional linkage studies, which have not yet yielded positive localization results. These types of studies, utilizing founder populations and mutations, are not unique to breast cancer genetics, and are being used successfully to understand other diseases.

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The Predictive Value of *BRCA1* and *BRCA2* Mutation Testing

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ABSTRACT

Genetic testing for mutations in *BRCA1* and *BRCA2*, two genes predisposing to breast and ovarian cancers, is available to women with a relevant family history. The aim of this study was to estimate the positive and negative predictive value of clinical sequence analysis of these genes. A reference graph showing positive and negative predictive values over a range of pre-test risk was derived, taking into account the sensitivity and specificity of a full-sequence analysis test. High positive and negative predictive values were found for women with pre-test risk between 4% and 40%, a range of risk commonly seen in clinical testing. The predictive value of full sequence and single site analysis of *BRCA1* and *BRCA2* therefore compares favorably with other diagnostic medical tests. Our results provide a numerical estimate of the predictive value of *BRCA* testing, and as such, provide a valuable tool to healthcare providers and families as they interpret *BRCA1* and *BRCA2* test results.

INTRODUCTION

Advances in molecular biology have led to the development of numerous genetic tests, but for these to be applicable in a clinical setting, it is important to carefully develop guidelines for selecting those patients most likely to benefit. This requires an assessment of the pre-test probability that an individual carries a deleterious mutation, and sufficient information so that health care workers are able to interpret the meaning of a positive or negative result.

There are more than 175,000 new cases of breast cancer and 24,000 cases of ovarian cancer each year in the US. Approximately 43,000 women die of breast cancer annually (American Cancer Society, 1998). Mutations in *BRCA1* (Miki et al., 1994) and *BRCA2* (Wooster et al., 1995; Tavtigian et al., 1996) are believed to account for approximately 5-10% of breast and ovarian cancer cases (Claus et al., 1996; Ford et al., 1998). Although mutations are rare (Ford et al., 1995; Peto et al., 1999), and risk estimates vary, female mutation carriers face up to an 87% risk of breast cancer and a 44% risk of ovarian cancer by age 70 years (Ford et al., 1994; Struewing et al., 1997; Ford et al., 1998; Hopper et al., 1999). Genetic tests are currently available to women with evidence of a family history of breast and/or ovarian cancers, to determine whether they carry deleterious mutations in *BRCA1* or *BRCA2* (Myriad Genetics Inc, 1996; Nelson, 1996). In the current study, we explore the interpretation of genetic testing in *BRCA1* and

BRCA2, in which the full sequence of the protein-coding regions and adjoining non-coding regions of these genes is examined.

MATERIALS AND METHODS

Risk estimates

In order to provide examples of prior probability estimates for a sample of family histories, the model described in Frank et al (Frank et al., 1998) was applied. This model was obtained by logistic regression analysis applied to data from 238 women with either breast cancer before age 50 years or ovarian cancer at any age, and at least one first or second degree relative with either diagnosis. The dichotomous variables retained in the final model were: presence of a relative with ovarian cancer; presence of a relative with male breast cancer; bilateral cancer in the consultand; breast cancer below 40 years in the consultand, and below 50 years in one relative. The results are shown in Table 1.

Evaluation of the genetic test

A Bayesian approach was applied to derive the positive predictive value (PPV) and negative predictive value (NPV) of *BRCA* testing (Galen et al., 1975). Sensitivity - the probability of a positive test in a mutation carrier, specificity - the probability of a negative result in a person who is mutation-free, and the individual's pre-test probability of carrying a mutation are required for the calculations. The pre-test probability is the prior probability of interest. It is the

conditional probability of a mutation in *BRCA1* or *BRCA2*, given family history and age at diagnosis.

The calculations are shown below, where p is the pre-test probability of a deleterious mutation, S is the clinical sensitivity of the test, and E is the clinical specificity of the test.

$$PPV = \frac{S \cdot p}{S \cdot p + (1-E) \cdot (1-p)} \qquad \text{Eq. 1}$$

$$NPV = \frac{E \cdot (1-p)}{E \cdot (1-p) + (1-S) \cdot p} \qquad \text{Eq. 2}$$

Mutations in *BRCA1* and *BRCA2* were analyzed by dye-primer sequencing as described in Frank et al. (Frank et al., 1998). They were considered deleterious if they resulted in a premature stop codon, an amino acid substitution in a functional domain, or if they caused aberrant splicing. It is estimated that 5-15% of deleterious *BRCA* mutations may be due to large deletions that are not detected by dye-primer sequencing. In the absence of a known mutation in the family, the clinical sensitivity of the full sequence analysis assay was therefore estimated to be 0.85. Clinical specificity was assumed to be >0.9995, since all positive results are repeated by the laboratory for confirmation, and the laboratory's validation studies have determined that the analytical specificity of the dye-primer sequencing method approaches one.

A curve was plotted to show the relationship between prior risk of mutation and predictive value across the whole spectrum of risk (Figure 1). A look-up table was also created for reference (Table 2).

Software.

Splus 2000 (Mathsoft Inc., Seattle, Washington) was used to generate the predictive value curves.

RESULTS

Table 1 illustrates the PPV and NPV for individuals with a range of family history information, as taken from Frank et al. (Frank et al., 1998). Patients with a prior probability of 25% of carrying a deleterious mutation have a greater than 99% chance of having a mutation in *BRCA1* or *BRCA2* if their test result is positive. A negative test in such an individual rules out the presence of a mutation with greater than 95% certainty. For higher prior probabilities, the PPV of the test increases towards 100%.

If the prior probability of a mutation is high, however, the NPV decreases. For example, a negative test result for a patient with 71% pre-test probability of carrying a mutation should be interpreted with caution. The patient still has a 27% probability of carrying a mutation. Nevertheless, her probability of being mutation-free has risen from 29% to 73%. Figure 1 shows how predictive value varies across the spectrum of prior risk and values are tabulated in Table 2.

DISCUSSION

The primary concern in the evaluation of any screening test is the occurrence of false negative results, leading to inappropriate reassurance, and false positive results, leading to unnecessary anxiety and treatment. ASCO guidelines suggest that genetic testing be considered for individuals with a prior probability of greater than 10% of carrying a deleterious mutation (ASCO, 1996). For individuals with prior risk in the 10-40% range, full sequence analysis gives PPV and NPV greater than 0.99 and 0.90, respectively. Sequence analysis of *BRCA1* and *BRCA2* in these individuals is therefore an accurate indication of mutation status.

The predictive value of *BRCA1* and *BRCA2* analysis is even greater for relatives of known mutation carriers who are tested only for the mutation carried by their relative. Clearly, any close relative of a known mutation carrier has a substantial pre-test probability of carrying the same mutation, and in our experience, over 48% of them test positive. The clinical and analytical sensitivity and specificity are greater than 0.99 for a previously characterized mutation, making full sequence analysis a highly effective tool for these cases.

Another group with elevated risk consists of women of Ashkenazi descent. Three mutations (*BRCA1* 185delAG and 5382insC, and *BRCA2* 6174delT) account for the majority of all those found in this group. *BRCA1* 185delAG is found in 20% of Ashkenazi Jewish women with breast cancer diagnosed before

age 42 years and *BRCA2* 6174delT accounts for 8% of cases (Neuhausen et al., 1996; Offit et al., 1996). Thirty-one percent of clinical samples from Ashkenazi breast cancer cases and 23% of those from Ashkenazi women without either breast cancer or ovarian cancer were found to carry one of these three mutations. This is a group for which relatives of known carriers should be tested for all three predisposing mutations, not just the one found in their relative. Despite their elevated risk, however, these women still fall within a range for which testing has strong positive and negative predictive power. The remaining clinical subgroups have a lower prevalence of mutations and the same test characteristics apply.

While we have used the model of Frank et al. (Frank et al., 1998) to derive estimates of the prior risk of mutation, our approach is applicable to other available models. Shattuck-Eidens et al. (Shattuck-Eidens et al., 1997) used logistic regression analysis to show that age, ethnicity, diagnosis and family history are all significant factors in determining a woman's risk of carrying a deleterious mutation in *BRCA1*. Couch et al. (Couch et al., 1997) also developed a predictive model to determine the odds that a women carries a mutation in *BRCA1* based on family history. Berry et al. (Berry et al., 1997) derived similar results in relation to *BRCA1*, and proposed a modification to take into account the likely effects of *BRCA2*. They found that the number and relationships of unaffected relatives, together with their current ages or ages at death were also critical determinants of carrier probability. This model would be expected to give higher

prior probability estimates than those of Frank et al. (Frank et al., 1998), because it is based on any deleterious changes, not just those detected by sequence analysis. Nevertheless, as shown in Table 2, modest changes in the estimate of prior risk do not alter the conclusion that sequence analysis has high positive and negative predictive values for most patients studied.

With the information in this analysis, health care workers can counsel patients about their individual risk following testing for mutations in *BRCA1* and *BRCA2* in a clinical setting. This may help to dispel misconceptions about the meaning of positive or negative tests in certain individuals. While it does require an assessment of prior risk for each individual, the result is a meaningful interpretation of the genetic testing results. This will permit patients and health care workers to make better-informed decisions as to how to proceed.

ACKNOWLEDGMENTS.

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Table 1. Examples of positive and negative predictive values of full-sequence analysis of *BRCA1* and *BRCA2* for women over a range of pre-test risk*

Consultand	Family history	Pre-test probability	PPV	NPV
Woman with breast cancer, dx < 50yr	One relative with breast cancer, dx < 50yr	0.25	0.98	0.95
Woman with breast cancer, dx < 50yr	One relative with ovarian cancer	0.35	0.98	0.92
Woman with breast cancer, dx < 40 yr	One relative with breast cancer, dx < 50 yr	0.40	0.99	0.91

Woman with breast cancer dx < 50 yr	One relative with either breast cancer dx < 50 yr, or ovarian cancer	0.59	0.99	0.82
Woman with breast cancer dx < 50 yr, with bilateral BC or ovarian cancer	One relative with ovarian cancer	0.71	0.99	0.73
Woman with breast cancer dx < 40 yr, with bilateral BC or ovarian cancer	One relative with ovarian cancer	0.89	1.00	0.45

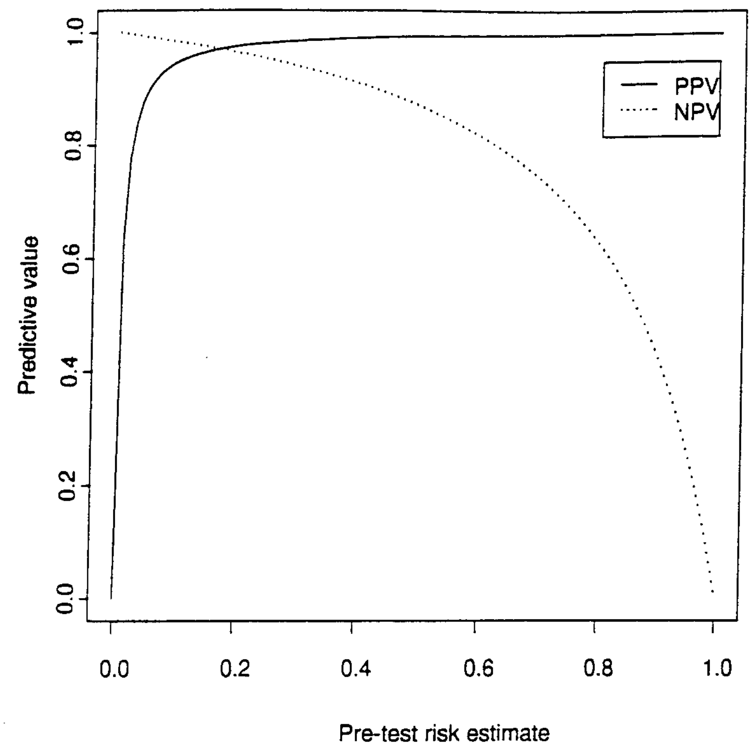
* Pre-test risks were calculated using the model of Frank et al. (Frank et al., 1998)

Table 2. Positive and negative predictive values of full-sequence analysis of *BRCA1* and *BRCA2*, in the absence of a known mutation in the family, for a range of prior probabilities

Pre-test probability	Positive predictive value	Negative predictive value
0.05	0.90	0.99
0.10	0.95	0.98
0.15	0.97	0.97
0.20	0.98	0.96
0.25	0.98	0.95
0.30	0.99	0.94
0.35	0.99	0.92
0.40	0.99	0.91
0.45	0.99	0.89
0.50	0.99	0.87
0.55	1.00	0.84
0.60	1.00	0.82
0.65	1.00	0.78
0.70	1.00	0.74

0.75	1.00	0.69
0.80	1.00	0.64
0.85	1.00	0.54
0.90	1.00	0.42
0.95	1.00	0.26

Figure 1. Positive and negative predictive values of full-sequence analysis of *BRCA1* and *BRCA2*. The positive (solid line) and negative (dashed line) predictive values are shown for a positive and negative test result, respectively, for a full sequence *BRCA* test in an individual whose family's mutation status is unknown. These curves are based on an assumed sensitivity of full sequence analysis of 85% and a specificity of 99.95% (see text for details). For individuals whose pretest probability of a mutation is between 10 and 40%, the predictive value of both positive and negative tests are high. For an individual whose pretest probability of mutation exceeds 50%, a positive test can be interpreted with absolute confidence, *i.e.*, the individual has the mutation. A negative test in such an individual leaves open the possibility that there may be an inherited familial mutation not detected by the test.



Predictive value
0.0
0.2
0.4
0.6
0.8
1.0
PPV
NPV
0.0
0.2
0.4
0.6
0.8
1.0
Pre-test risk estimate

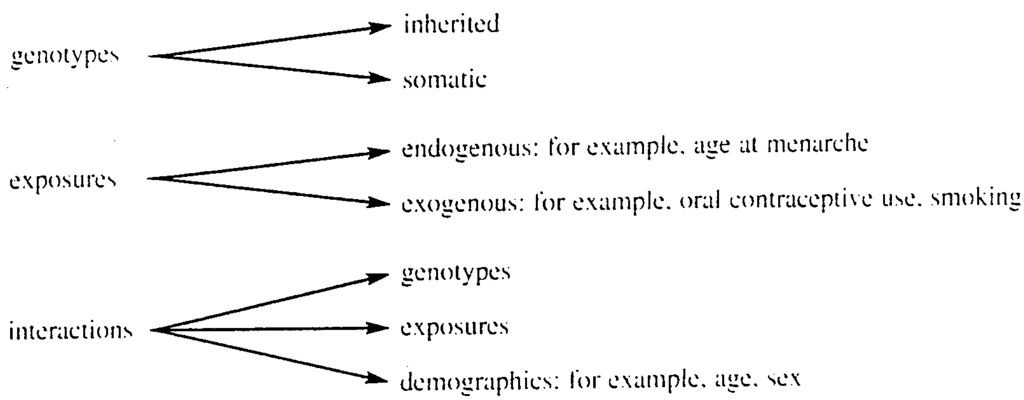
Susan L. Neuhausen

GENETIC EPIDEMIOLOGY OF BREAST, OVARIAN AND ENDOMETRIAL CANCERS LOW PENETRANCE GENES

INTRODUCTION

Cancer results from complex interactions among genetic, hormonal, growth and environmental factors. In genetic epidemiological studies, important considerations are those factors, both genetic and epidemiological/lifestyle which may explain the etiology or progression of the disease (figure 2). Endometrial, breast and ovarian cancers are all hormone-related cancers. Therefore, factors which affect the biosynthesis, secretion and metabolism of estrogen should be examined. Epidemiology/lifestyle factors include smoking, diet, reproductive characteristics such as age at menarche and at menopause, nulliparity, use of oral contraceptives and hormone replacement therapy. A family history of cancer is an important risk factor. Relative risks range from 2 to 9 depending on type of cancer, age and number of first-degree relatives affected by the disease [1]. Segregation analyses of pedigrees often suggest a genetic basis for the family history. Genetic factors include rare genes

Figure 2 - Factors important in genetic epidemiological studies of etiology of cancer.



which confer a high-risk for developing the disease and common genes which confer a low risk, such as genes which metabolize carcinogens or which encode growth factors, enzymes and receptors that regulate hormones and are involved in cellular proliferation. In this chapter, an overview of genes, which may confer a lower risk of developing cancer (low penetrance), yet be more common in the population, is presented. These low penetrance genes may act alone or may be in response to environmental or lifestyle triggers, for example, smoking, high-fat diets, oral contraceptive use.

EPIDEMIOLOGICAL RISK FACTORS

ENDOMETRIAL CANCER

The primary risk factors for endometrial cancer are largely associated with "unopposed estrogens", where no progesterones are present [2,3]. Obesity, diabetes, hypertension and nulliparity are commonly associated conditions [4]. Family history has also been reported to be a risk factor. In a case-control study of family history of endometrial cancer in first degree relatives with a median age of cancer at 61 years, the odds ratio was 1.5 [5]. The odds ratio was 2.8 in a case-control study of women between the ages of 20-54 years [6]. Gruber et al [6] suggest that nearly 5 per cent of incident endometrial cancer among women ages 20-54 years may be due to a family history of endometrial cancer and 2 per cent may be related to colorectal cancer. This confirms a report of Sandles et al [7] in which they suggest that a portion of familial risk is due to HNPCC and a separate portion to endometrial cancer alone. Endometrial cancer is the most frequent extracolonic cancer in HNPCC [8] and 2 per cent of endometrial cancer is related to colorectal cancer [6].

OVARIAN CANCER

Hormonal risk factors commonly associated with ovarian cancer are nulliparity, early age at menarche and late age at menopause [9,10]. A family history of ovarian cancer is a major risk factor, with relative risks ranging from 2.0-4.3 for first-degree relatives [11-14]. There is familial aggregation of ovarian cancer, and breast, endometrial and colon cancers [15,16]. The endometrial and colon cancer associations are largely due to HNPCC and the ovarian and breast syndrome due to *BRCA1*. In an unselected population of ovarian cancer cases, approximately 10 per cent had *BRCA1*, *BRCA2* or HNPCC mutations [17].

BREAST CANCER

For breast cancer, known reproductive factors such as age at menarche and menopause, age at first pregnancy, number of full-term pregnancies, and oral contraceptive use are important risk factors [18,19]. A family history of breast cancer has been identified as a major risk factor for the development of breast cancer with estimates of a 2-10 fold increased risk to first-degree relatives of a breast cancer case [20-24]. Claus et al [25] reported that age at onset is the strongest indicator of familial risk of breast cancer. In a study comparing the incidence of familial breast cancer among non Hispanic Caucasians, African Americans, and Hispanic breast cancer cases, 10 per cent of non Hispanic Caucasian women and 14.9 per cent of African American women reported a first-degree relative with breast cancer as compared to 2 per cent of Hispanic women [26]. Hispanic women in the US have lower rates of breast cancer. Part of the reason for the low rate of breast cancer in Hispanics may be because they lack the risk associated with a family history.

A genetic predisposition to breast cancer may explain a large proportion of early-onset breast cancer. Estimates are that 7 per cent of breast cancer cases and 10 per cent of ovarian cancer cases in the general population are due to breast cancer susceptibility genes [27], and that approximately one-third of breast cancer cases diagnosed between 20-29 years are caused by mutations in high-penetrance breast cancer susceptibility genes [27]. *BRCA1* and *BRCA2*, two genes which confer susceptibility to developing cancer are discussed in the chapter *Genetic Epidemiology of Hereditary Breast, Ovarian and Endometrial Cancer.* Not all women with deleterious *BRCA1* or *BRCA2* mutations will develop cancer. Estimates of the age-specific risk attributable to mutations range from 56 to 80 per cent [28,29]. Even among individuals and families who share common founder mutations, large differences exist in ages of onset of breast cancer and in relative incidence of breast and ovarian cancers [30-32]. It is possible that mutation carriers in high-risk families have a greater risk, not just because they are gene carriers, but because they also inherited other lower-penetrant risk genes. Peto et al [33] suggest that only a small proportion of familial risk of breast cancer in families with few cases of cancer is attributable to mutations in *BRCA1* and *BRCA2*. They hypothesize that the remaining genes conferring susceptibility are of lower risk. In the general population, which includes those individuals with no family history to a strong family history, the high penetrance, rare genes such as *BRCA1* and *BRCA2* appear to explain even a smaller proportion of breast cancer.

COMMON GENES WHICH MAY PLAY A ROLE IN CANCER

The possible role of inherited (germ-line) mutations in common genes in the etiology of breast, endometrial, and ovarian cancers is the focus of this chapter. These cancers are all hormone-dependent and therefore, genes which confer risk for one cancer may also confer risk for the others. A general description of these common genes in comparison to rare genes such as *BRCA1* and *BRCA2* is shown in table I.

Why are we interested in studying genes which confer a low risk of cancer yet are common in the population? Identification of relevant genes important in the occurrence and/or progression of breast, ovarian and endometrial cancers could provide clues for the design of better preventative and treatment strategies. The objective of many genetic epidemiological studies is to provide information that could lead to predictive individual risk assessment and to aid in decision-making regarding screening, preventive surgeries, chemotherapeutic drug strategies and lifestyle choices.

Table 1 - General classes of genes causing susceptibility to disease.

class	variant frequency	absolute risk	attributable risk	examples
rare genes	low	high	low	*BRCA1, BRCA2*
common genes	high	low	high	*GSTM1, CYP1A1, COMT*

Which genes are likely candidates? One needs to study the significant epidemiological risk factors for the clues they provide to discern possible genetic etiologic pathways in breast, ovarian, and endometrial cancers. As breast cancer is the most common of the three cancers, most of the examples presented are in breast cancer. However, many, if not most of these genes, may be relevant to endometrial and ovarian cancers as well.

SUGGESTIVE ETIOLOGIES/ PATHWAYS FROM EPIDEMIOLOGICAL STUDIES

From epidemiological studies, a number of demographic, reproductive and hormonal factors have been reported to influence risk of breast and ovarian cancers [34]. Many of the risk factors for breast cancer reflect cumulative exposure of breast tissue to estrogens. From case-control studies, a number of reproductive factors has been associated with increased breast cancer risk, for example early age at menarche, late age at first birth, nulliparity/low parity, and late age at natural menopause [18,19,35]. One of the strongest risk factors for breast cancer is age at first pregnancy, where risk is doubled in a woman whose first full-term pregnancy occurs after age 29 years compared with before age 20 years. Other reproductive and hormone-related factors such as low parity and early age at menarche also confer increased risks of breast cancer. Among postmenopausal women, late age at natural menopause is implicated as a risk factor. For ovarian cancer, nulliparity and few pregnancies have been related to a higher risk [10,36], and early menarche, late age at natural menopause, late age at first pregnancy, and infertility confer a moderately increased risk [9,37]. Thus, exposure to endogenous female sex hormones appears to play a role in the etiology of breast and ovarian cancers. The association between breast cancer risk and exogenous hormone use is less clear [38]. In a collaborative study of 54 epidemiological studies of breast cancer, a relative risk for breast cancer of 1.3 was observed for current oral contraceptive users [39]. White et al [40] reported a modestly increased risk with long-term OC use among young women. OCs are associated with a 50 per cent decreased risk of ovarian cancer [9,10]. For HRT, an increased risk of breast cancer has been observed with long durations and recent use [41], although other studies have shown no association [42-44].
Reproductive factors have also been examined in *BRCA1* and *BRCA2* mutation carriers. In one study of *BRCA1* and *BRCA2* mutation carriers, parity was associated with significant differences in age-specific risk of breast cancer, consistent with results from population studies. There was no effect of age at first or last pregnancy [45]. There was a significant correlation between age at diagnosis and age at last birth for ovarian cancer. Narod et al [46] reported similar findings in a study utilizing some of the same mutation carriers as in Goldgar et al [45]. There was an increased risk of breast cancer associated

with low parity and with recent birth cohort. The risk of ovarian cancer decreased with increasing age at last childbirth and increased with increasing parity. These results indicate that the risk of cancer among *BRCA1* and *BRCA2* mutation carriers is modified by endogenous hormones and that risk factors are similar to those in the general population. *BRCA1*-mRNA expression studies showed that gene expression is induced during puberty, pregnancy and after treatment of ovariectomized animals with 17β-estradiol and progesterone [47]. This implies a role for *BRCA1* in the differentiation of breast and other tissues which is triggered by (exogenous or endogenous) hormones. Both puberty and pregnancy may represent periods of increased susceptibility to carcinogenesis in the breast in *BRCA1* carriers. Based on the results of these studies, there likely are hormonal and genetic factors which modulate age-specific and overall incidences of breast and ovarian cancers in mutation carriers. Thus, the putative low penetrance genes discussed in this review are also applicable to individuals with known mutations predisposing to cancer.

GENES INVOLVED IN CELL PROLIFERATION

Genes in the estrogen biosynthesis and metabolism pathways

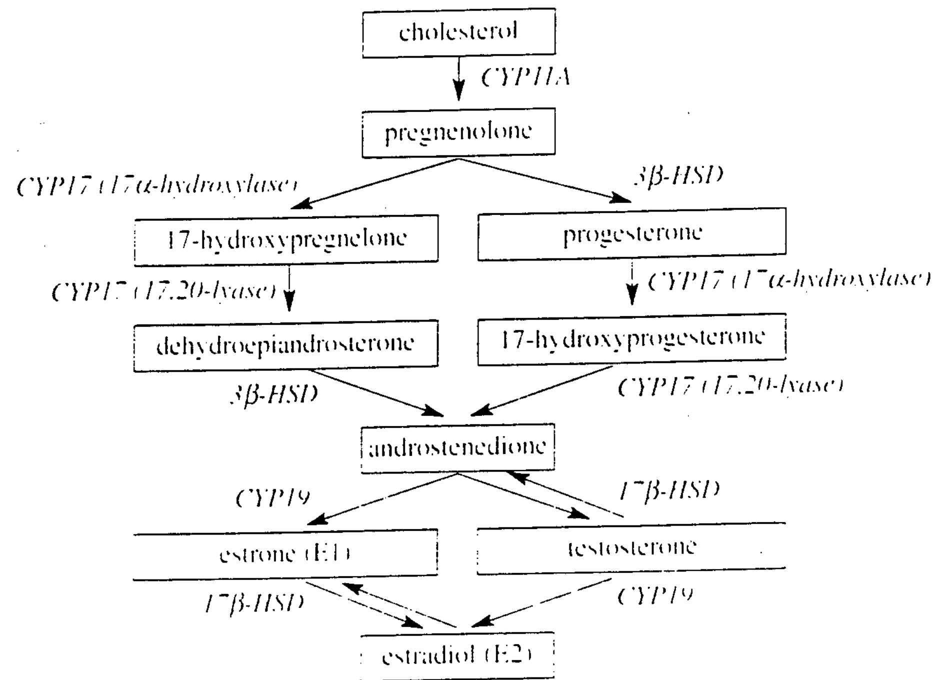
The link between estrogen, particularly 17β-estradiol (E2) and breast, ovarian and endometrial cancers, possibly through its role in stimulating cell proliferation, has long been known. In a prospective study of postmenopausal women, serum estrogen levels were measured. In general, those women who subsequently developed breast cancer had higher serum levels of estrone, total estradiol and free estradiol (for all three, $p= 0.06$) and a lower percentage of estradiol bound to SHBG ($p <0.01$) than in women who had not developed cancer [48,49]. In a second prospective case-control study, there was a significantly increased risk for breast cancer in women in the highest quartile for bioavailable estradiol (RR= 3.6) and for free testosterone (RR= 3.3) as compared to the lowest quartile [50]. Therefore, enzymes which regulate estradiol biosynthesis and metabolism may be important for breast cancer development. Polymorphisms which alter activity of the proteins produced from these genes may be important risk factors for hormonally-regulated cancers by regulating the level of circulating estrogen.

Estrogens are generated from the conversion of androgens, which are generated from cholesterol through a series of reactions (figure 3). Therefore, variants in genes involved in synthesis of androgens and estrogens are potential risk factors for these cancers through their actions in regulating and altering hormonal levels. Important enzymes for synthesis include the cytochrome *p450* genes (*CYP11A*, *CYP17* and *CYP19*) [51]. Aromatase (*CYP19*) is the key enzyme converting androgens to estrogens and is generally considered the rate-limiting step [52]. As a result, it has a major role in regulating estrogen levels. Estrone is the primary steroid produced. It is converted to estradiol (E2), a more biologically potent estrogen, by 17 β-hydroxysteroid dehydrogenase type I (*HSD17B1*).

Aromatase is present in both normal and tumor cells in the breast [53]. Within tumors, intratumoral aromatase appears to be over-expressed and is important in converting circulating androgens to estrogens, probably in association with 17β-HSD type I and estrogen sulfatase [52,54,55]. In population-based studies, increased risks of developing breast cancer have been associated with a polymorphism in *CYP19* [56,57].

PolyCystic Ovarian Syndrome (PCOS) disrupts normal ovarian function and increases the risk of endometrial cancer [58] and the risk of ovarian cancer 2.5-fold [59]. The

Figure 3 - Pathway of estrogen synthesis.

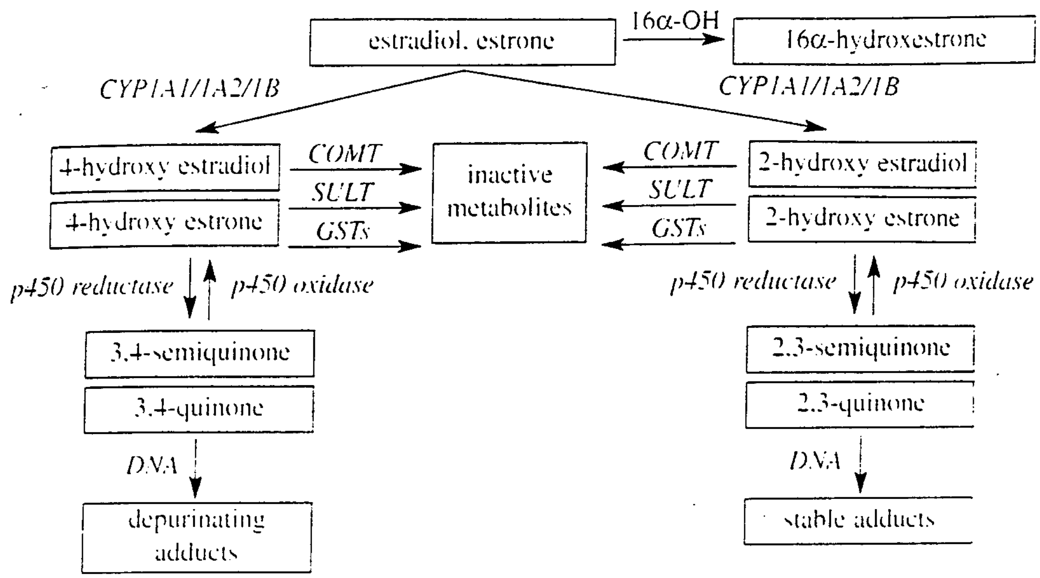


hyperandrogenism of PCOS appears due to *CYP17* which regulates androgen synthesis, and is itself regulated by the insulin/IGF system [60,61]. In a study examining relatives with and without PCOS, an A2 variant in *CYP17* was associated with PCOS with an odds ratio of 2.20 [62]. Techatraisak et al [63] reported no differences in the allele frequencies between PCOS patients and a control population and thus no increased risk for PCOS. Serum levels of estradiol, measured in premenopausal, ovulating women, were significantly higher in women with A2 alleles as compared to women homozygous for the wildtype A1 allele, suggesting genetic control of serum hormone levels by *CYP17* [64]. A similar result was reported when estrogens were measured in postmenopausal women [65]. In one study, the A2 *CYP17* variant was associated with an increased risk of metastatic disease (2.52, 95 per cent CI, 1.07-5.94) [66]. However, this result was not confirmed in subsequent studies [65,67-69].

A further effect of estrogen on breast cancer risk may be from metabolism of estrogens. The pathway for metabolism of estrogens is shown in figure 4. Estradiol is oxidized primarily at C-2, but also at C-4 to form the 2-3- and 3-4 catechols. There are data to suggest that 16α-hydroxyestrone, estrogen catechols, and the estrogen quinones from oxidation of the catechols are genotoxic [51,70]. 16α-hydroxyestrone is believed to cause excess cell proliferation and to cause DNA damage. The E2 and E1 catechols are intermediates in the production of more reactive semiquinones and quinones. Semiquinones and quinones serve as substrates for redox cycling and have the potential to cause oxidative stress and damage that may contribute to the development of cancer. The catechols are inactivated by Catechol-O-MethylTransferase (*COMT*), as well as by glucuronidation and sulfation.

CYP1A1 is a primary enzyme in metabolism of estradiol and estrone to catechol

Figure 4 - Pathway of estrogen metabolism.



estrogens. There have been several population-based studies examining the association of *CYP1A1* genetic polymorphisms with breast cancer risk. Ambrosone et al [71] reported an increased risk of breast cancer in postmenopausal women associated with the Val462Ile polymorphism, whereas Rebbeck et al [72] found no significant association. A significant association of a *MspI CYP1A1* polymorphism with increased risk of breast cancer in African-American women, but not in Caucasian women, was reported [73,74]. In a prospective study from the Nurses' Health Study, there was no overall increase in breast cancer risk associated with two polymorphisms in *CYP1A1* [75]. However, there was an increase in breast cancer risk among women with variant alleles who had started smoking before age 18 years as compared to non smokers who were homozygous wild type [75]. In a study of *BRCA1* and *BRCA2* mutation carriers, the risk of breast cancer in premenopausal mutation carriers with at least one 3' *MspI* A2 variant was significantly reduced (OR= 0.58, p= 0.04) [76]. In a case-control study of endometrial cancer, the *CYP1A1* variant showed a strong association with endometrial cancer risk (OR= 6.36, 95 per cent CI 1.99-26.5) [77], as did two other *CYP1A1* polymorphisms in a smaller study (OR= 3.67, 95 per cent CI 1.21-13.26) [78].

COMT plays an important role of converting catechol estrogens to inactive metabolites which are water soluble and excreted in urine [51]. A genetic variant in *COMT*, which confers lower enzyme activity [79] was examined in a case-control study of breast cancer cases [80]. There was a significant increase in risk in postmenopausal women with a BMI > 24.47 kg/m^2 and in postmenopausal women with the glutathione s-transferase mu (*GSTM1*) null variant or a *GSTP1* variant [80]. *GSTs* and SulfoTransferases (SULTs) also convert the 2- and 4-hydroxy estradiols and estrones to inactive metabolites. The *GSTs* are discussed below in the section *Carcinogen Metabolism Genes*. The ability to convert the catechol estrogens into inactive metabolites may be important for reducing breast cancer risk.

Steroid hormone receptors

Variants in the ER and PR genes which affect expression or binding may be important risk factors for these hormonally-regulated cancers. Overexpression of estrogen receptors in normal breast tissue increases breast cancer risk (OR= 3.16, 95 per cent CI 1.89-5.28) possibly by enhancing estrogen sensitivity [81]. Anderson et al [82] screened for mutations in ER and reported a Gly160Cys polymorphism in ER that may be associated with breast cancer risk, but more studies are needed. Parl et al [83] reported that breast cancer cases with tumors homozygous for the 0.7 kb PvuII fragment in ER were significantly younger (mean age of diagnosis at 50.4 years) than those either heterozygous or homozygous wildtype (mean age of 64.4 and 64.6 years, respectively). A mutation in the PR gene has been associated with an increased risk of ovarian cancer [84]. However, in two subsequent studies, this association was not present for either breast or ovarian cancer [85,86].

Androgens play a role in regulating processes in breast, ovarian and endometrial tissue. The Androgen Receptor (AR) is expressed in normal ovarian epithelial cells and is down-regulated in most ovarian cancer cells [87]. In another study, AR were present in 90 per cent of 94 epithelial ovarian cancer cells [88]. AR is also expressed in normal endometrium [89] and in endometrial cancer [90]. AR is expressed in epithelial cells in normal breast tissue [91], as well as in some tumor breast tissue where it mediates breast tumor growth and progression [92], and may be coexpressed with ER and PR [91]. A CAG repeat in AR (AR-CAG) is inversely associated with the level of transcriptional activation of AR [93,94]. This polymorphism has been examined as a risk factor for breast cancer. In a population-based case-control-family study of women less than 40 years at diagnosis of breast cancer, there was no association between the length of the CAG repeat and breast cancer [95]. In a study of 304 women with germ-line *BRCA1* mutations, 165 with breast cancer and 139 without cancer, the effect of the AR-CAG repeat length on breast-cancer penetrance was evaluated. Women who carried at least one AR allele with ≥ 28, ≥ 29, ≥ 30 CAG repeats were diagnosed with breast cancer 0.8, 1.8, and 6.3 years earlier than women who did not carry at least one such allele [96]. This suggests that androgen signaling pathways may be important in modifying development of cancer, at least in *BRCA1* associated cancers.

Another important steroid hormone receptor may be the Vitamin D Receptor (VDR) which exerts influence in breast epithelial cells [97] by mediating the action of 1,25(OH)2D3, which is involved in cell proliferation and differentiation in breast cancer cells [98-101]. In two studies, the majority of breast cancer tumors expressed VDR [102,103]. Colston et al [103] reported that there was significantly longer disease-free survival in those patients with VDR-positive tumors. Eisman et al [104] found that VDR protein levels were associated with lymph node metastases. In a recent study, the association of a VDR polymorphism with breast cancer was examined [105]. There was no association with risk of developing breast cancer, but for breast cancer cases absent the *TaqI* site, there was a significantly increased risk for lymph node metastasis (OR= 1.8) [105]. This suggests that variants in VDR may confer an increased risk for breast cancer progression (as measured by metastases). VDR expression has been observed in endometrial carcinoma tissue [106] and in ovarian cells of mammals (hens) and birds [107].

Insulin-like growth factor pathway genes

In population-based studies, obesity has been reported as a risk factor for breast,

ovarian and endometrial cancers [2,108, 109]. Obesity results in Significantly Increased Insulin (INS) levels, as well as increased levels of Insulin-Like Growth Factor I (IGF1) and estrogen [110]. Estradiol, IGF1 and insulin appear to stimulate proliferation in normal breast epithelium and increase breast cancer risk [111-113]. Thus, genes in the growth factor signalling pathway are good candidates to study for their roles in the etiology of these cancers. Genes in the IGF signalling pathway also likely function as regulators of steroid hormone actions in the endometrium [114].

The estrogen and growth factor signalling pathways are interrelated in that INS, IGF, SHBG, estrogen and their receptors are involved in regulation of each other [115-117]. Insulin itself is a potent mitogen [118]. The INS 5' VNTR is associated with levels of insulin gene expression both in vivo and in vitro [119,120], as well as with levels of IGF2 [121]. The effect of insulin may be greater through down-regulating Insulin Growth Factor Binding Proteins (IGFBPs) [113]. A decrease in IGFBPs would increase bioavailability of IGF1 and IGF2, which are potent mitogens that regulate proliferation of breast cells [122,123]. IGFBP-3 binds IGF1 and modulates the activity of IGF1 [124], and higher circulating levels of IGF1 and lower levels of IGFBP-3 have been reported in breast cancer patients [113,125]. IGFBP-3 may also play an IGF-independent role in growth regulation of cancer cells by directly inhibiting growth [126]. IGFBP-3 levels have been associated with poor prognostic features (tumor size and low ER) [130]. IGF2 has been implicated in regulation of breast cancer cell growth [127-129].

IGF1 may be an important breast cancer risk factor because of its role in cell growth and differentiation, as well as its effect on ER activity [115,131] and PR activity [132,133]. IGF1 levels are increased during periods of more rapid growth, such as puberty [123]. Ninety percent of breast tumors are insulin receptor-positive and over-express IGF1 [111]. In a prospective case control study, there was a strong association between circulating IGF1 serum concentrations and the risk of breast cancer in premenopausal women, especially for those women less than 50 years of age at the time of blood collection [134].

SHBG may be regulated by IGF1, because as IGF1 levels increase, SHBG levels decrease [135-137]. SHBG binds to testosterone and estradiol, thereby regulating the biologically available levels of these hormones [138]. Higher levels of SHBG would reduce free levels of estrogen, thereby protecting against the development of breast cancer. SHBG has been observed in both normal and cancerous breast tissue by immunostaining [139].

IRS1 is the major cytoplasmic substrate of the insulin and IGF1 receptors in most insulin sensitive tissues, including breast tissue. Nolan et al [140] observed that IRS1 was critical in control of growth of MCF-7 cells and in cell survival. High IRS1 expression is a factor in shorter disease-free survival in patients with small tumors (< 2 cm) [116, 130]. This suggests that IRS1-mediated signalling is involved in growth regulation in breast tumors [130,141].

The growth factors binding proteins and receptors of the IGF pathway are important in ovarian follicle growth and development, as they both stimulate ovarian cellular mitosis and steroidogenesis and inhibit apoptosis [142]. IGF1 plays a role in proliferation of ovarian cancer and appears to interact with estradiol to regulate growth [143,144]. PCOS was described above, and the action of *CYP17*, which regulates androgen synthesis, appears to be regulated by the insulin/IGF system [60,61].

The IGF system likely functions to mediate steroid hormone action in the endometrium as well as in the breast and ovaries. Rutanen et al [114] examined levels of the IGFBPs in normal and cancerous endometrial tissue and observed that IGFBP-1

expression was suppressed in cancerous tissue, suggesting that an excess stimulation of cells by IGFs leads to uncontrolled proliferation. The role of insulin in growth of endometrial cancers was studied in five endometrial cancer cell lines [145]. Insulin stimulated cell growth of all the cell lines, possibly through both direct action as a mitogen and indirectly through the IGF pathway. Insulin suppresses and progesterone induces IGFBP expression in the endometrium, so that suppression of IGFBP-1 may explain the increased risk of endometrial cancer when there is hyperinsulinemia or unopposed estrogens (absence of progesterone) [114]. Kleinman et al [146,147] investigated the effects of estradiol and tamoxifen on the IGF system in Ishikawa endometrial cancer cells. They found that estradiol and tamoxifen sensitize the cells to the effects of IGFs by elevating IGF1R levels and decreasing IGFBP levels.

CARCINOGEN METABOLISM GENES

The final set of genes to be discussed are those which metabolize carcinogens, both exogenous and endogenous. Polycyclic Aromatic Hydrocarbons (PAHs), which are common in urban environments and present in tobacco smoke, are possible human breast carcinogens. They are lipophilic and stored in adipose tissue [148], are metabolized and activated by human mammary epithelial cells [149] and cause mammary tumors in rodents [150]. PAHs are metabolized by phase I enzymes, including the cytochrome *p450* enzymes (for example, *CYP2E1*, *CYP1A1* and *CYP2D6*) and *NAD(P)H*: Quinone Oxidoreductase (*NQO1*), into reactive intermediates which are then detoxified by phase II enzymes, including Glutathione S-Transferases, GSTs (for example, *GSTP1*, *GSTM1*, *GSTT1*) [151] and Epoxide Hydroxylase (EPHX) [152].

Polymorphisms in these enzymes may affect breast cancer risk because of alterations in the metabolization of the PAHs. Several researchers have conducted studies examining the effects of genetic variants in *CYP1A1* and *CYP2D6*, which may enhance the conversion of PAHs to reactive intermediates and of the null (poor metabolizer-PM) phenotype of *GSTs*, whereby detoxification of PAH intermediates may not occur. The studies of *CYP1A1* were described previously. Ladero et al [153] compared individuals who were homozygous for a *CYP2D6* variant to phenotypically normal individuals and reported a relative risk of 2.09 for breast cancer. Wolf et al [154] and Pontin et al [155] saw no increased risk associated with this variant.

GSTs may be important in breast cancer because of their role in detoxifying exogenous carcinogens in the breast and/or by detoxifying the catechol estrogens to inactive metabolites. However, several studies report no significant association between breast cancer and the null phenotype of *GSTM1* [71,156,157]. In contrast, in a recent study, breast cancer risk was increased in *GSTM1* null individuals who were ≥ 50 years of age, suggesting that it may play a role in postmenopausal breast cancer development [158]. Rebbeck et al [156] examined *GSTT1* in breast cancer cases with a family history and reported that mean age at diagnosis was significantly earlier in *GSTT1*-null carriers. This result suggests that women who carry *GSTT1* null alleles may be more susceptible to the effects of PAHs than women who carry normal alleles, because they are unable to form inactive metabolites and therefore have higher exposure levels, which results in development of breast cancer at an earlier age. In a study of endometrial cancers, there was no risk associated with either the *GSTM1* null or the *GSTT1* null alleles [78].

N-AcetylTransferase (NAT) is important in the acetylation of arylamine carcinogens. It

is one of the major enzymes in breast tissue that activates the aromatic and heterocyclic amines for which the main exposure is cigarette smoking or consuming well-done meat. Increased risks of breast cancer have been reported in women who smoke and who are NAT2 slow acetylators [159] or who have a *CYP2E1* variant [160]. In a recent case control study of postmenopausal women, a NAT1 polymorphic allele was associated with an approximately 4-fold increased risk of breast cancer and was elevated in those that smoked and those who consumed well-done meat [161].

Although not an enzyme which metabolizes carcinogens, the *HRAS1* VNTR (Variable Number Tandem Repeat) has been associated with an increased risk of breast cancer in case-control studies [162] and an increased risk of ovarian cancer in *BRCA1* mutation carriers [163]. There are likely other genetic polymorphisms which have been reported to confer an increased risk of breast cancer which are not described in this review.

CONCLUSIONS

In this chapter, we have described genes and their protein products which could confer an increased risk for development of cancers of the breast, ovary and endometrium. Because these genes likely confer only a small risk for developing cancer, large cohorts and case-control populations are required in order to assess their significance. It may be that it is gene-gene and gene-environment interactions that are important for risk of developing these cancers. Based on studies looking at main effects and interactions, the size of interaction effects are often larger than main effects because of synergistic effects of factors. The role of low penetrance genes in breast, ovarian and endometrial cancers is still an under-explored research area.

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Prevalence of *BRCA2* mutations in male breast cancer cases
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Introduction: Male breast cancer is rare, with an incidence rate of 0.5-1/100,000 per year. The objective of this grant is to study unselected male breast cancer cases to estimate the attributable risk of male breast cancer due to *BRCA2* mutations.
Materials and Methods: This study was approved by the University of Utah Institutional Review Board. Male breast cancer cases were recruited primarily through the Utah Cancer Registry, as well as from the Wyoming Cancer Registry, Dr. Bishop at the ICRF in the UK, a support group on the internet, and Dr. Offit at Memorial Sloan Kettering Cancer Center in New York. For each participant, a 15 ml blood sample and a self-administered questionnaire with detailed family history of breast and other cancers were collected. DNA was extracted from blood using a Gentra™ kit. Single strand conformation polymorphism (SSCP) analysis followed by sequencing of variants was performed to identify mutations in coding regions and intron/exon boundaries of *BRCA2*. SSCP was performed on 73 amplicons, with an average size of 250 base pairs.
Results: 141 Caucasian male breast cancer cases are participating. Age at diagnosis ranges from 28-93 years. Of the 94 cases with family history data, 52% have a family history of breast cancer in at least one first degree relative. One individual had three variants, two silent and 1 missense (all likely polymorphisms). Four missense mutations of unknown functional significance, 3 mutations that are likely polymorphisms, and seven obvious polymorphisms were identified. Ten frameshift mutations were found in 15 cases, including 5 cases with the 6174delT Ashkenazi Jewish founder mutation. Based on mutations known to be deleterious, the prevalence is 10.6% (15/141). This is a conservative estimate, because we do not yet have data on all samples for all amplicons. Five mutation carriers have a positive family history, 2 have a negative family history, and 7 are unknown.
Conclusions: The percentage of *BRCA2* mutations in this sample is 10.6%. Accounting for the sensitivity of SSCA of approximately 80%, the population prevalence is 13.3% [(15/141)/.80]. Family history is not a good predictor of *BRCA2* mutation status. *BRCA2* mutations appear to be more prevalent in unselected male cases than in unselected female breast cancer cases.